Effects of High Frequency Electrical Stimulation on Pain Mechanisms in Humans

An Honours Thesis

Submitted to the Faculty of Psychology and Exercise Sciences

in partial fulfilment of the requirements for the

Honours Degree of Bachelor of Arts in Psychology

in

The School of Psychology

by

Shirley Ming Lui Kwok

This thesis is presented for the Honours degree of Bachelor of Arts in Psychology of Murdoch University, and submitted in October 2013. I declare that this thesis is my own work and all sources of information used in this thesis have been fully acknowledged.

Name: Shirley Ming Lui Kwok

Signature: _____

Date: 21-Oct-2013

COPYRIGHT ACKNOWLEDGEMENT

I acknowledge that a copy of this thesis will be held at the Murdoch University Library.

I understand that, under the provisions s51.2 of the Copyright Act 1968, all or part of this thesis may be copied without infringement of copyright where such a reproduction is for the purposes of study and research.

This statement does not signal any transfer of copyright away from the author.

Signed: ……...

Full Name of Degree: Honours Degree of Bachelor of Arts in Psychology
e.g. Bachelor of Science with Honours in Chemistry

Thesis Title: Effects of High Frequency Electrical Stimulation on Pain Mechanisms in Humans

Author: Shirley, Ming Lui, Kwok

Year: Oct-2013

General Acknowledgments

First, I would like to extend my gratitude to my supervisor, Professor Peter Drummond. I would not have completed this thesis without his knowledge and guidance. Second, I am also grateful to PhD. students, Lechi Vo and Aries Suhnan, who spent extra time helping me understand all the testing equipment and procedures from the beginning. Last, I would like to thank the laboratory technician, Man Trac, who was always there to help me fix the technical issues when I was doing testing with my participants.

TABLE OF CONTENTS

Abstract

The purpose of this study was to explore effects of high frequency electrical stimulation (HFS) on pain mechanisms among healthy individuals. Findings suggested that HFS produced hyperalgesia (increase in sensitivity) on the conditioned forearm and analgesia (decrease in sensitivity) on the forehead and feet. These findings suggest that three underlying pain mechanisms (peripheral sensitisation, central sensitisation, and diffuse noxious inhibitory controls) are affected by HFS. However, analgesia to pressure-pain on the ipsilateral (to the HFS conditioned forearm) forehead was absent in the present study. Also, blink reflex activity elicited by large surface electrodes, as an electrophysiological measurement to study central processing of nociception and sensitisation, did not respond to HFS conditioning. Thus, no corresponding result of HFS conditioning was observed between psychophysical response and blink reflex activity. Several factors, might be contributing to these non-significant findings, were discussed at the end.

Effects of High Frequency Electrical Stimulation on Pain Mechanisms in Humans

The World Health Organization (1946) defines health as "…a state of complete physical, mental and social wellbeing, not merely the absence of disease of infirmity". From our experience, we know that these three factors (physical, mental and social wellbeing) contributing to good health are generally not independent from each other. Especially from a biopsychosocial perspective, good health can be largely affected by the interaction among biological, psychological and social factors. For people who are experiencing chronic pain or suffering pain disorders, it is more likely that their physical condition would also have an adverse effect on their mental functioning and social wellbeing. Chronic pain has become a serious public issue and many studies have confirmed that chronic pain has impacts on psychological health, daily activities, employment and financial wellbeing (Blyth et al., 2001; Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006; Gatchel, 2004; Schopflocher, Taenzer, & Jovey, 2011). For instance, a large-scale telephone survey was undertaken among 15 European countries and 19% of 46,394 respondents (aged between 18 and 81 years old, mean age of participants was 49.4 years) reported suffering chronic pain (the pain had lasted over six months and they had experienced pain in the last month and several times during the last week prior to the survey) (Breivik et al., 2006). Moreover, subsequent in-depth interviews with them showed that 21% of 4,839 respondents had been diagnosed with depression because of their pain, which also caused other interference with their daily activities, such as unable to work outside the home or losing their job (Breivik et al., 2006). This is just one of many chronic pain research studies in industrialised countries showing that pain is for many people in different age groups and it can have negative impacts on many aspects of a person's life (Blyth et al., 2001; Bouhassira, Lantéri-

Minet, Attal, Laurent, & Touboul, 2008; Breivik et al., 2006; Schopflocher et al., 2011; Verhaak, Kerssens, Dekker, Sorbi, & Bensing, 1998).

Therefore, trying to understand the abnormal pain mechanisms, which are causing the chronic pain symptoms, is important in order to develop efficacious treatments for physical distress related to pain disorders. However, before we could do that, we need to have a thorough understanding of the normal pain mechanisms in order to compare to, and understand the abnormal pain mechanisms. Thus, one focus of the pain-related research has been on clarifying the pain mechanisms among healthy people, such as trying to understand that how pain signals are initially detected, transmitted and perpetuated, what are the factors could influence people's experience of pain and how these mechanisms would change when different types of stimulation (such as mechanical or heat stimulation) presented at different body areas.

Pain Mechanisms

Pain can vary in intensity (such as mild, moderate, or severe), quality (such as sharp, burning, or dull), duration (such as transient, intermittent, or persistent) and referral (such as superficial, deep, localised, or diffuse), and there are four primary types of pain: nociceptive pain (caused by noxious peripheral stimuli), inflammatory pain (inflammation caused by tissue damage at the cellular level), neuropathic pain (caused by peripheral nerve damage or spinal cord injury), and functional pain (abnormal processing of the nervous system) (Woolf, 2004).

Historically, several theories were proposed to explain pain. For example, the specificity theory suggested that specific pain receptors in body tissue projected to a pain centre in the brain. In contrast, the pattern theory suggested that stimulus intensity and central summation were the critical determinants of pain; whereas later, the gate control theory suggested that the spinal cord contained a neurological "gate" that

modulated the flow of pain signals to the brain (Melzack & Wall, 1965). Some of the more recently identified pain mechanisms will be discussed later, such as the peripheral sensitisation, central sensitisation and analgesia, and diffuse noxious inhibitory controls (DNIC).

Nerve Fibres

Before I could discuss some specific pain mechanisms, there is a need to briefly describe the peripheral nerve fibres in our skin. Generally, there are three types of peripheral nerve fibres, the sensory nerve fibres (afferent fibres), the motor nerve fibres (efferent fibres), and the autonomic nerve fibres (autonomic fibres) (Landon, 1976). Afferent neurons carry nerve impulses from receptors or sense organs towards the central nervous systems whereas efferent nerves carry nerve impulses away from the central nervous system to effectors such as muscles or glands (Landon, 1976; Saylor, 2011). Afferent neurons terminate in the dorsal horn of the spinal cord, which is the first site of synaptic transfer in the nociceptive pathway(Costigan & Woolf, 2000).

The peripheral nerve fibres can be classified into three major groups based on their diameter: the A group, the B group and the C group (Human Anatomy, 2011; Landon, 1976). There are four major types of A group fibres: A-alpha (α), A-beta (β), A-gamma (γ) and A-delta (δ) fibres, but as only A-beta and -delta fibres carry sensory information such as touch, temperature and pressure they are the main focus in pain-related research (Boddunan, 2010). A-beta fibres are large myelinated sensory neurons that detect innocuous stimuli applied to skin, muscle and joints and they generally do not contribute to pain whereas thinly myelinated A-delta fibres detect nociceptive and thermal stimuli (Julius & Basbaum, 2001; Scholz & Woolf, 2002). The myelin layer of A group nerve fibres increases the speed at which impulses carrying sensory information propagate along the fibres, but for unmyelinated C group fibres

(transducing the nociceptive and thermal stimuli), impulses move continuously as waves and more slowly compared to the myelinated fibres (Julius & Basbaum, 2001; Scholz & Woolf, 2002). Therefore, we usually feel sharp and acute sensations first (impulses travelling fast by A group fibres), and then the delayed diffuse dull sensations (impulses travelling less fast by C group fibres).

Primary and Secondary Hyperalgesia

The current study largely relates to two pain mechanisms: hyperalgesia (increase in sensitivity to painful stimuli) and analgesia (decrease in sensitivity to painful stimuli).

Hyperalgesia is defined as "a state of increased intensity of pain sensation induced by either noxious or ordinarily non-noxious stimulation of peripheral tissue" (Hardy, Wolff, & Goodell, 1950, p. 115). In order to better understand different pain conditions caused by hyperalgesia, chemical, thermal, mechanical and electrical experimental models applied to different areas in human skin have been developed to investigate different pain symptoms induced by different stimuli and mechanisms (Ali, Meyer, & Campbell, 1996; Baumann, Simone, Shain, & LaMotte, 1991; Culp, Ochoa, Cline, & Dotson, 1989; Kilo, Schmelz, Koltzenburg, & Handwerker, 1994; Magerl, Fuchs, Meyer, & Treede, 2001; Raja, Campbell, & Meyer, 1984; Sumikura, Andersen, Drewes, & Arendt-Nielsen, 2006; Vo & Drummond, 2012, 2013).

Two types of hyperalgesia have been identified: primary and secondary hyperalgesia. Primary hyperalgesia, also referred to as peripheral sensitisation, occurs at the site of injury area and is characterised by a lowered pain threshold and increased pain sensibility to both heat and mechanical stimuli (such as pinprick, brush, punctate or blunt stimuli) (Culp et al., 1989; Hardy et al., 1950; Kilo et al., 1994; LaMotte, Shain, Simone, & Tsai, 1991; Raja et al., 1984). In general, primary hyperalgesia is thought to

be due to an enhanced responsiveness or sensitisation of the nociceptors (the A-delta and C-fibres) that signal pain. Common ways to evoke primary or secondary hyperalgesia in animals or in humans are using laser thermal stimulator or electrical stimulation with low frequencies (0.5 to 2 Hz) or with bursts of very high frequency (100 Hz) (Klein, Magerl, Hopf, Sandkühler, & Treede, 2004; Klein, Stahn, Magerl, & Treede, 2008; Sluka, Judge, McColley, Reveiz, & Taylor, 2000; Vo & Drummond, 2012, 2013). For example, in order to study primary hyperalgesia, Meyer, Ringkamp, Campbell, and Raja (2005) induced a controlled heat injury on the hand and recorded the neural activity from nociceptors (A-fibres and C-fibres) that responded to both mechanical and heat stimuli and they found that burn injuries caused sensitisation of both A-fibres and C-fibres.

On the other hand, secondary hyperalgesia, also referred to as central sensitisation, occurs in undamaged skin adjacent to the site of injury and is characterised by increased pain sensibility to mechanical stimuli (but not heat stimuli) (Ali et al., 1996; LaMotte et al., 1991; Raja et al., 1984). For example, Raja, Campbell, and Meyer (1984) used a laser thermal stimulator to apply heat stimuli and harmless burns to two spots (20 mm apart) on the skin of participants' hand and found that hyperalgesia to the pinprick mechanical stimuli was present at the site of burn (primary hyperalgesia) and also in the surrounding area (secondary hyperalgesia), but hyperalgesia to the heat stimuli occurred only at the site of the burn. The findings indicated that the characteristics of primary and secondary hyperalgesia may differ and they also suggested that the mechanisms for hyperalgesia to mechanical and heat stimuli may differ as well (Raja et al., 1984). More recent research has demonstrated that sensitisation of the peripheral nociceptors does not account for secondary hyperalgesia; rather, it is due to changes in the central processing of nociceptive information (Meyer

et al., 2005). Moreover, two types of secondary hyperalgesia to mechanical stimuli have been observed, one is to light touch, also referred to as allodynia, and the other one is to punctate stimuli (Meyer et al., 2005; Ziegler, Magerl, Meyer, & Treede, 1999). For instance, Ziegler et al. (1999) conducted a study to investigate the relative contributions of A- and C-fibres to secondary hyperalgesia and found that, when A-fibres were blocked by pressure at the wrist while C-fibres were still unaffected, the capsaicin-induced pain for healthy participants was equal in magnitude to the pain produced without a nerve block but no hyperalgesia to punctate stimuli could be detected. After the block had been released, punctate hyperalgesia was found to be fully developed. Thus, they suggested that secondary hyperalgesia to punctate stimuli was induced by nociceptive C-fibres discharge but mediated by nociceptive A-fibres.

Analgesia

In contrast to hyperalgesia, analgesia refers to a decreased sensitivity usually observed remotely from a painful stimulus. It used to be called counter-irritation phenomenon, which is the "paradoxical pain-relieving effects of pain elicited from heterotopic body areas" (Willer, Roby, & Bars, 1984, p. 1096). An interesting example could be using cold-induced pain applied to the hand to relieve dental pain (Melzack, Guite, & Gonshor, 1980). This phenomenon, known as the diffuse noxious inhibitory controls (DNIC) refers to the inhibited activity of pain-signalling neurons in the spinal dorsal horn and in trigeminal nuclei by noxious stimuli applied to body areas far remote from the excitatory field of these neurons (Kakigi, 1994; Knudsen & Drummond, 2009, 2011; Lautenbacher, Roscher, & Strian, 2002; Schliessbach et al., 2012; Villanueva, Peschanski, Calvino, & Le Bars, 1986; Vo & Drummond, 2012, 2013).

Recently, cold- or electrical-induced limb pain has consistently demonstrated a decrease in sensitivity to pressure-pain sensations in the forehead especially on the same

side of the affected limb (Knudsen & Drummond, 2009, 2011; Vo & Drummond, 2012, 2013). For example, Knudsen and Drummond (2009) investigated the effect of unilateral limb pain on sensitivity to pain on each side of the forehead of healthy participants and showed that analgesia to pressure pain was greater in the ipsilateral forehead. Also, Vo and Drummond (2013) investigated whether healthy participants' analgesic response is induced by ultraviolet B radiation (UVB, triggers signs of peripheral sensitisation) or high frequency electrical stimulation (HFS, triggers signs of both peripheral and central sensitisation) and demonstrated that ipsilateral forehead analgesia developed after HFS but not UVB conditioning. This suggests that ipsilateral analgesia was not due to peripheral sensitisation because it failed to develop after UVB conditioning.

Vo and Drummond (2012) have suggested one possible explanation for this ipsilateral pain analgesia that, previous research has showed that electrical stimulation of nociceptive fibres (A-delta and C fibres) could trigger a pain inhibitory mechanism descending from the locus coeruleus (LC) in the brain and this pain inhibitory mechanism suppresses nociceptive activity at all segmental levels of the spinal cord via noradrenergic projections (Hitoto, Tsuruoka, Hiruma, & Matsui, 1998; Rahman, D'Mello, & Dickenson, 2008). In experiments on rats, Tsuruoka, Hitoto, Hiruma, and Matsui (1999) found that unilateral hindpaw inflammation produces an increase of the noradrenaline level from the LC in the dorsal horn ipsilateral, but not contralateral, to the site of inflammation, which suggest that descending modulation from LC was active only ipsilaterally to the inflamed paw (Tsuruoka, Matsutani, & Inoue, 2003; Tsuruoka & Willis Jr, 1996; Tsuruoka & Willis, 1996). Therefore, it is possible that the same mechanism produces the ipsilateral forehead analgesia in humans.

Blink Reflex

The blink reflex, as a non-invasive way of studying trigeminal transmission in humans, can be an objective electrophysiological measurement to study central processing of nociception and sensitisation. Using electrical or laser radiant heat stimulation of the trigeminal supraorbital nerve (which courses across the forehead) is one common way to elicit blink reflexes in humans (Blumenthal et al., 2005; Ellrich, Bromm, & Hopf, 1997; Ellrich & Treede, 1998; Giffin, Katsarava, Pfundstein, Ellrich, & Kaube, 2004; Vo & Drummond, 2012).

The blink reflex evoked by electrical stimulation usually consists of an early, ipsilateral to the stimulated site R1 component with an onset latency of 11 ms and two late, bilateral R2 and R3 components, with onset latencies of 33 and 84 ms, respectively (Ellrich & Hopf, 1996; Hopf, 1994; Kimura et al., 1994; Rossi, Risaliti, & Rossi, 1989). Previous research has investigated the individual components of the blink reflex by selectively activating the nociceptive fibres (such as the A-delta and C fibres). For example, Ellrich et al. (1997) conducted a study to investigate whether the R2 was mediated by activation of tactile or nociceptive afferents. And the result showed that both R1 and R2 were evoked by innocuous stimuli but only the R2 was also evoked by selective activation of nociceptive fibres. Further evidence shows that R2 is mainly mediated via wide dynamic range (WDR) interneurons (which can be activated by innocuous and noxious mechanical stimuli) and R1 is relayed by low threshold mechanoreceptive (LTM) neurons (which respond just to innocuous mechanical stimuli) (Ellrich & Treede, 1998; Willis, 2004). The R3 component of the blink reflex is mainly evoked by the cutaneous A-beta stimulation rather than a nociceptive response (Ellrich & Hopf, 1996; Rossi et al., 1989; Téllez, Axelrod, & Kaufmann, 2009). Therefore, pain-related research generally focuses on the R2 component of blink reflex and the

outcomes indicate that the activities of R2 latency and its integrated amplitude (area under the curve or AUC) depend on stimulus type and intensity (Ellrich & Hopf, 1996; Ellrich & Treede, 1998; Kaube, Katsarava, Käufer, Diener, & Ellrich, 2000; Kaube et al., 2002). For example, Kaube et al. (2000) compared two types electrodes to elicit blink reflexes and demonstrated that the modified concentric electrodes (designed to enable high current density at low current intensity and selectively activated superficial nociceptive fibres), evoked R2 component only, but not R1.

Aims and Hypotheses

This study addressed the following research questions: 1. Does HFS produce hyperalgesia on the conditioned forearm? 2. Does HFS produce analgesia to pressure-pain on the ipsilateral forehead? 3. Does HFS produce analgesia to pressure-pain on the ipsilateral foot? 4. Does HFS produce inhibition of ipsilateral blink reflexes elicited by large surface electrodes?

Forearm

The first aim of this study was to compare sensitivity changes in the primary and secondary areas to sharpness, heat and pressure-pain on the conditioned forearm before and after the HFS conditioning. Based on past research (Culp et al., 1989; Hardy et al., 1950; Vo & Drummond, 2012), it was hypothesised that HFS would produce hyperalgesia to mechanical (sharp and pressure-pain) and heat stimuli in the primary area of the conditioned forearm. Also, as demonstrated by previous research related to the development of secondary hyperalgesia (Ali et al., 1996; Raja et al., 1984; Vo & Drummond, 2013), it was hypothesised that HFS would produce hyperalgesia to mechanical stimuli, but not to heat stimuli in the secondary area of the conditioned forearm.

Forehead

The second aim was to compare the sensitivity changes of both sides of the forehead before and after the HFS conditioning on the forearm. It was expected to see a bilateral analgesia on both sides of the forehead due to the diffuse noxious inhibitory controls (DNIC) phenomenon. Particularly, as discussed earlier, we expected to observe the development of the ipsilateral reduction in pain due to the HFS-triggered ipsilateral pain inhibitory mechanism descending from the locus coeruleus (LC), which had been established in previous research (Knudsen & Drummond, 2009, 2011; Vo & Drummond, 2012, 2013). Thus, it was hypothesised that HFS conditioning would produce analgesia to pressure-pain stimuli on the ipsilateral forehead.

Foot

The third aim was to compare the sensitivity changes of the foot before and after HFS conditioning on the forearm. As we expected to see similar changes on the feet due to the same pain mechanisms determining sensation changes on the forehead, it was hypothesised that HFS conditioning would produce analgesia to pressure-pain stimuli on the ipsilateral foot.

Blink Reflex

Vo and Drummond (2012) conducted a study to investigate changes in body sensations and blink reflexes elicited by concentric electrodes after HFS conditioning on the forearm. Interestingly, they found a dissociation between forehead ipsilateral (to HFS conditioned forearm) analgesia to pressure-pain and ipsilateral facilitation of blink reflexes after HFS conditioning (i.e. the integrated amplitude (area under the curve or AUC) of R2 ipsilateral to HFS conditioning was significantly greater than the contralateral side AUC). They attributed this dissociation to the different nerve fibres activated by the algometer (testing the pressure-pain threshold) and concentric

electrodes (eliciting blink reflexes) that the algometer activated deeper nerve fibres (A-beta fibres) but, according to Kaube et al. (2000), only superficial nociceptive A-delta and C-fibres (but not A-beta fibres) would be activated by concentric electrodes.

Therefore, instead of using concentric electrodes, the current study used large surface electrodes (26x34 mm) and a stronger current level (7.5 mA) to elicit blink reflexes in order to activate the A-beta nerve fibres in deeper skin layers (Kaube et al., 2000). A large skin-electrode contact area was to ensure that an adequate amount of current would flow into the stimulated skin area and according to Kaube et al. (2000), current level beyond 2 mA would result innocuous stimulation of A-beta fibres. Thus, by using the large surface electrodes to activate deep nerve fibres, we expected to observe an inhibitory effect of HFS on blink reflex activity, contradicting to the facilitatory effect of HFS when using concentric electrodes to evoke blink reflexes.

Thus, the last aim of the current study was to compare the excitability of blink reflexes elicited ipsilateral and contralateral to the HFS conditioned forearm. The excitability of blink reflexes was measured in terms of the onset latency and the AUC of R2 component of blink reflexes. Based on the ipsilateral pain inhibitory mechanism discussed above and our expectation that HFS would produce an inhibitory effect, it was hypothesised that the onset latency of R2 for blink reflexes that were ipsilateral both to supraorbital stimuli (blink reflex stimulation) and the conditioned forearm site (HFS stimulation) would be longer (reacting slower) than the latency to the contralateral HFS conditioned side. Also, it was hypothesised that R2 AUC for blink reflexes that were ipsilateral both to supraorbital stimuli and the conditioned forearm site would be smaller (reacting less in amplitude) than AUC to the contralateral HFS conditioned side, indicating a development of an ipsilateral inhibitory effect of HFS conditioning.

Method

Participants

The participants were nine males and 17 females aged between 18 and 51 years, recruited from the Murdoch University School of Psychology. They were awarded credit points for their participation. Exclusion criteria included pregnancy, breastfeeding, acute or chronic pain, diabetes, heart disease, epilepsy or other long-term medical condition. Participants gave their written informed consent for the procedures, which were approved by the Murdoch University human research ethics committee.

Procedures

All experiments were conducted by the same experimenter (SK) in a laboratory (maintained at 21 ± 1°C) at Murdoch University. Participants sat in a comfortable armchair throughout the experiment.

One ventral forearm (right or left side) area was assigned as the test site and the laterality of the test site was counterbalanced across participants to minimise the order effect. To minimise skin electrical resistance, the participant was asked at the beginning of the experiment to gently clean the test site (with a pumice stone), which was then rinsed with water and dried with paper towel.

Psychophysical tests. Sensations were investigated at the test site (ventral area of the forearm), the forehead and an outer side area of the dorsum (equidistant from the ankle and the toes) of both feet. For the forearm area, the test site was assigned as the primary area and an area 1 cm distal to the primary area was assigned as the secondary area. For the forehead, sensations were investigated on an equivalent area on both sides. Similarly, sensations were investigated on an equivalent area of both feet.

Rating scale. Participants reported pain, sharpness, or heat using a verbal rating scale ranging from 0 to 10. Zero indicated “no pain”, “not sharp”, or “not hot” at all and

ten indicated “extreme pain”, “extremely sharp”, or “extremely hot” depending on which sensation was being tested. Prior to conducting the baseline psychophysical tests, the participant was tested for few trials to differentiate ratings for various sensations.

Sharpness. To investigate sensitivity to mild sharpness, a 10 g von Frey’s monofilament (Neuro-pen, Owen Mumford, USA) was applied perpendicularly to the skin surface with sufficient pressure to bend the monofilament for one second. To measure sensitivity to more intense sharpness, a sharp tip (pinprick) with a calibrated spring mechanism exerting a force of 40 g (Neuro-pen, Owen Mumford, USA) was applied perpendicularly to the skin surface for two seconds.

Heat. To investigate sensitivity to heat, a 1.5 cm diameter metal probe heated to forty-four ± 0.2 °C was placed at the site for seven seconds.

Pressure pain threshold (PPT). To investigate sensitivity to pressure-pain, an algometer (FDX, Wagner Instruments, USA) with a modified 8 mm diameter hemispheric rubber tip was applied perpendicularly to the site at 100 g/second until the participant reported starting to feel pain.

To minimise order effects, the psychophysical tests were conducted with each stimulus (mild sharpness, more intense sharpness, heat, and pressure-pain) being applied in runs alternating between the primary and the secondary areas of the test site, between the two sides of the forehead, and between the feet. Also, the side tested first alternated between each side of the forehead, and between each foot in counter-balanced order across participants. To minimise effects of repeated resting, each test was performed only once in each round.

Blink reflex procedure. After the psychophysical tests, the participant was prepared for the blink reflex procedure. The stimulating electrodes were diagnostic resting ECG surface electrodes, (SKINTACT RT-74, 26x34 mm, Leonhard Lang Ltd,

UK). On each side of the forehead, a surface electrode was attached to the supraorbital region and a ground electrode was attached at the outer side of the surface electrode with a distance about 2 cm. The blink reflexes were recorded bilaterally using modified disposable Cleartrode electrodes (ConMed Corporation, USA) attached over the orbicularis oculi muscles of the lower eyelid and the outer corner of each eye and a ground electrode was attached behind the right ear. Electromyography signals were amplified with an electromyographic bio-potential amplifier (Biopac Systems, Inc., USA), digitized by an MP100 Biopac Systems Analogue/Digital Channel receptor at 2,000 Hz (Biopac Systems, Inc., USA) and displayed on a computer monitor using AcqKnowledge software (Biopac Systems, Inc., USA).

To elicit blink reflexes, two series of electrical stimuli were applied at a current of 7.5 mA. Each series consists of 10 monopolar square-wave electrical stimuli at 15-second intervals to minimise habituation. The stimulus was a 3-pulse train with 0.5 ms pulse duration, and an inter-pulse interval of 5 ms. Triple-pulse stimulation increases the sensation of pain and facilitates the R2 area under the curve (R2 AUC), and is thus more suited to examine nociceptive pathways than single pulses (Giffin et al., 2004). Participants rated pain on the 0-10 scale after each supraorbital stimulus. Within each series, an equal number of stimuli were administered on each side of the forehead. Stimulus administration alternated between sides such that no more than two stimuli were delivered sequentially on the same side in order to minimise habituation.

High frequency electrical stimulation (HFS). The HFS was applied to the primary area of the test site. The electrical stimuli were generated by a constant current stimulator (DS7A; Digitimer, Welwyn Garden City UK) and delivered via a custom-built electrode that consisted of 24 copper pins with 0.2 mm diameter tips mounted on a 2 cm x 3 cm perspex block such that the tips projected 0.5 mm from the surface of the

block. Electrodes with these properties preferentially activate superficial nociceptive A-delta and C fibres (Nilsson et al, 1997; Inui et al., 2000).

Electrical detection threshold (EDT). The EDT was determined first before applying the HFS conditioning, used the method of limits for two ascending and two descending sets of single pulses at 2 ms pulse width and an inter-pulse interval of five seconds (Vo & Drummond, 2013). The stimulus intensity, starting at 0.1 mA, increased in steps of 0.1 mA until the participant perceived the stimulus, and then decreased in steps of 0.05 mA until the stimulus was no longer perceived (Vo & Drummond, 2013). The procedure was then repeated. The EDT was defined as the mean of the four stimulus intensity levels.

After the EDT was determined for both arms, one-second burst of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times EDT up to a maximum 8.5 mA) was applied at the primary area on the test site. The participant rated pain on the 0-10 scale after the stimulation. Then, HFS conditioning was applied at the same area. This consisted of five one-second bursts of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times EDT up to a maximum 8.5 mA) with a nine-seconds rest between each burst (Klein et al., 2008; Lang et al, 2007). Five minutes later, another one-second burst of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times EDT up to a maximum 8.5 mA) was applied again at the same area. The participant rated pain again on the 0-10 scale after the stimulation.

After five minutes rest, the psychophysical tests were re-conducted at the forearm (primary and secondary areas), on each side of the forehead and on each foot. Finally, the blink-reflex procedure was repeated again, in which two series of supraorbital stimuli were administered as previously described (See Appendix A for the timeline of the procedures).

Data Filtering and Reduction

The electromyographic waveforms were filtered to remove 50 Hz electrical noise and frequencies below 20 Hz. For each blink reflex, the R2 rectified area under the curve (AUC) was measured between 27 and 87 ms after the stimulus onset (Ellrich & Treede, 1998). In addition, the R2 AUC of all blink reflexes before and after HFS conditioning was expressed as the percentage of the AUC of blink reflexes administered at baseline (before HFS conditioning) to compare the changes.

Statistical Analyses

Assessment of primary and secondary hyperalgesia. Changes in sensitivity to sharpness (mild and more intense), heat, and pressure-pain at the primary and secondary areas across time (before and after HFS conditioning) were investigated in repeated-measures analyses of variance (ANOVA). After all the repeated-measures analyses, student's *t*-tests were then used to investigate significant interactions by assessing changes in each sensation at the primary and secondary areas across time.

Forehead sensitivity. Changes in sensitivity to sharpness (mild and more intense), heat and pressure-pain between the two sides of the forehead and across time (baseline and after HFS conditioning) were investigated in repeated-measures analyses of variance.

Pain ratings to electrical stimuli on the forearm. Changes in pain ratings to the one-second burst of electrical stimuli at the primary areas across time (before and after HFS conditioning) were investigated in repeated-measures analysis of variance.

Blink reflex tests. Based on the laterality to the HFS-treated site and to the supraorbital stimulus, the blink reflexes were classified into "ii" (ipsilateral to both the HFS-treated site and supraorbital stimulus), "cc" (contralateral to both the HFS-treated site and supraorbital stimulus),"ic" (ipsilateral to the HFS-treated site and contralateral

to the supraorbital stimulus) and "ci" (contralateral to the HFS-treated site and ipsilateral to the supraorbital stimulus).

Pain ratings to supraorbital stimuli. Changes in pain ratings to supraorbital electrical stimuli across time (before and after HFS conditioning), and in relation to the laterality of the supraorbital stimuli (ipsilateral or contralateral to the HFS-treated site) were investigated in a repeated-measures analysis of variance.

Changes in latency. Onset latencies of R2 responses were measured for each stimulus after rectification of the raw waveform. Changes in R2 latency before and after HFS conditioning were investigated in a repeated-measures analysis of variance in relation to the laterality of the HFS conditioning (ipsilateral or contralateral to the HFS-treated site) and the laterality of blink reflexes (ipsilateral or contralateral to supraorbital stimulation). Student's *t*-test was used to explore interactions in relation to the latency changes before after HFS conditioning.

Changes in AUC. Changes in R2 AUC before and after HFS conditioning were investigated in a repeated-measures analysis of variance in relation to the laterality of the HFS conditioning (ipsilateral or contralateral to the HFS-treated site) and the laterality of blink reflexes (ipsilateral or contralateral to supraorbital stimulation). Student's *t*-test was used to explore interactions in relation to the AUC changes before and after HFS conditioning.

Results

Assumption Testing for ANOVA

Statistical analyses were carried out to test that the data complied with the assumptions that underlie ANOVA. Normality of scores (for each psychophysical test, R2 latency and R2 AUC before and after HFS) were first assessed using Shapiro-Wilk and boxplots; and extreme scores denoted by an asterisk were treated as outliers and

removed before running ANOVA analyses (see Appendix B for the list of the removed outliers). After removed the outliers, visual inspection of all the boxplots indicated that there were still deviations observed from the norm but none of them were severe departures from normality. Because ANOVA is quite robust against moderate violations of normality, those violations of norms were not considered to be a threat to the interpretation of parametric statistics.

Homogeneity of variance (for each psychophysical test, R2 latency and R2 AUC before and after HFS) was assessed by the F_{max} test (largest sample variance^2/smallest sample variance^2). According to Tabachnick and Fidell (2007), homogeneity of variance can be assumed when F_{max} is less than 10. Appendix C indicated that the homogeneity of variance assumption had not been violated for any test. The Sphericity assumption was not applicable for current analyses because each repeated-measures factor had only two levels.

Electrical Detection Threshold (EDT)

The EDT ranged from 0.75 mA to 1.05 mA (M = 0.43, SD = 0.21). The mean of pain ratings of HFS on the treated side was 5.21 (SD = 3.44), and no significant difference was found when comparing ratings before and after HFS conditioning.

Changes in Forearm Sensitivity after HFS conditioning

Sharpness sensitivity to von Frey's monofilament. After conditioning, sharpness sensitivity to von Frey's monofilament increased in both primary and secondary areas (main effect for Time $F(1, 23) = 4.43$, $p = .046$; main effect for Area $F(1, 23) = 1.20$, $p = .285$; Time x Area interaction $F(1, 23) = 0.75$, $p = .396$) (see Figure 1). Pain ratings of sharpness to von Frey's monofilament also increased after HFS conditioning in both primary and secondary areas (main effect for Time $F(1, 23) = 6.25$,

p = .02; main effect for Area $F(1, 23) = 3.71$, p = .067; Time x Area interaction $F(1, 23) = 1.15$, p = .295) (see Figure 2).

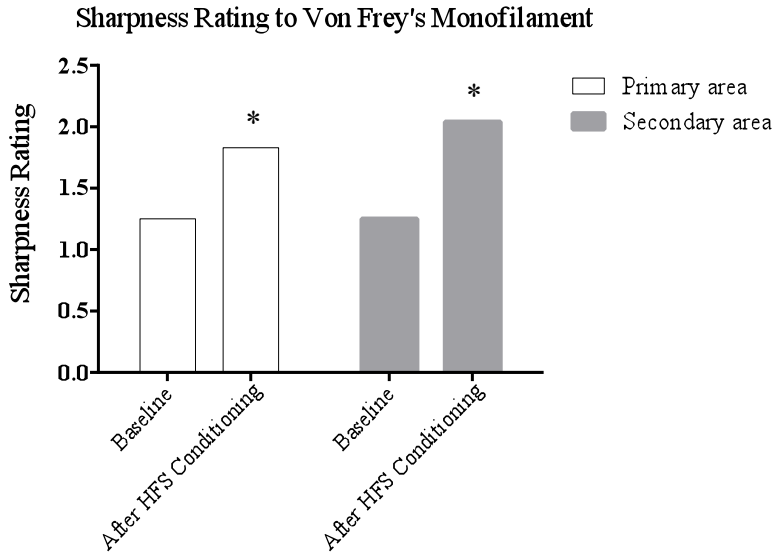


Figure 1. Sharpness rating to von Frey's monofilament in the forearm before and after HFS. *Sharpness ratings to von Frey's monofilament increased significantly after HFS in primary and secondary areas.

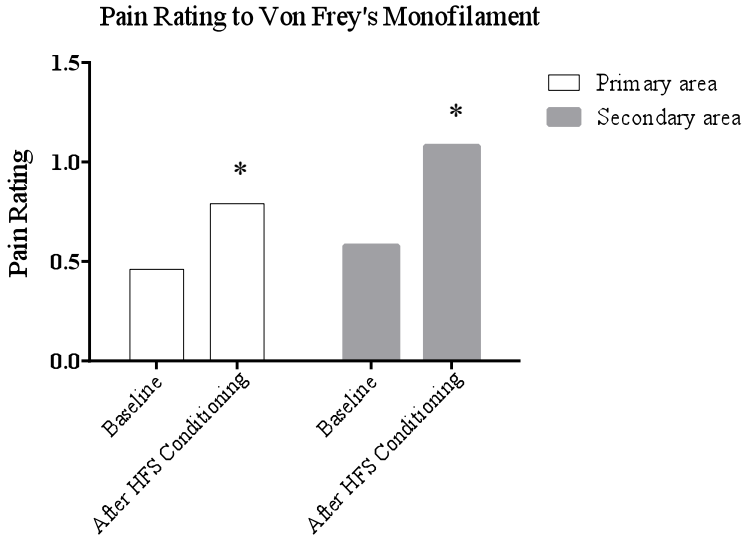


Figure 2. Pain rating to von Frey's monofilament in the forearm before and after HFS. *Pain ratings to von Frey's monofilament increased significantly after HFS in primary and secondary areas.

Sharpness sensitivity to pinprick. After conditioning, sharpness sensitivity to pinprick increased in both primary and secondary areas but none of the effects that

involved Time or Area was statistically significant (see Figure 3). Pain ratings of sharpness to pinprick also increased after HFS conditioning in both primary and secondary areas but none of the effects that involved Time or Area was statistically significant (see Figure 4; see Appendix D for the summary of all the non-significant results).

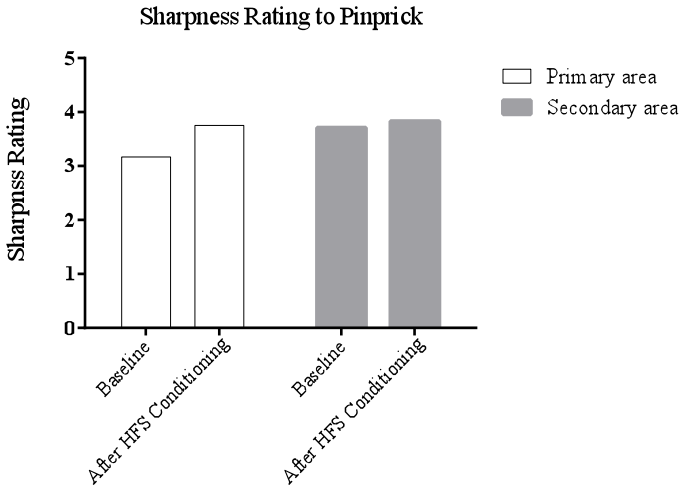


Figure 3. Sharpness rating to pinprick in the forearm before and after HFS.

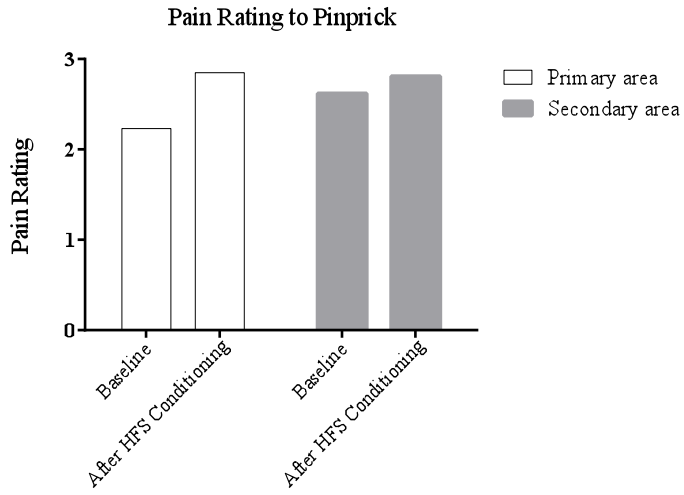


Figure 4. Pain rating to pinprick in the forearm before and after HFS.

Pressure pain threshold (PPT). After conditioning, PPT decreased (i.e. the sensitivity to blunt pressure increased) in both primary and secondary areas but none of the effects that involved Time or Area was statistically significant (see Figure 5).

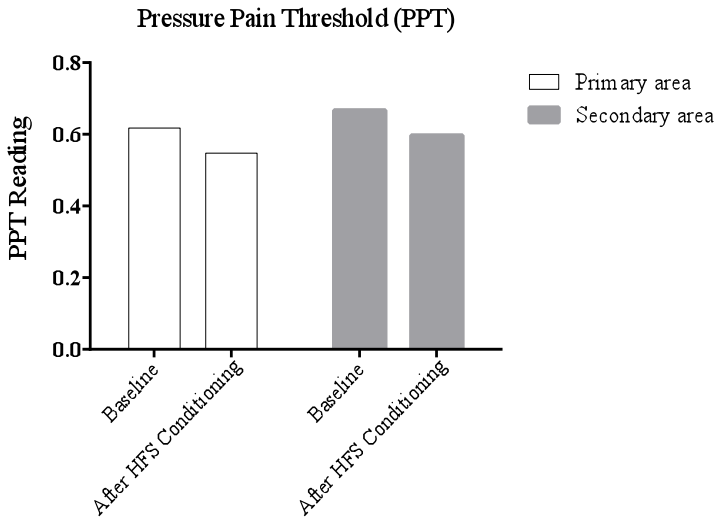


Figure 5. Pressure pain threshold changes in the forearm before and after HFS.

Heat sensitivity. After conditioning, heat sensitivity increased in the primary area and remained quite stable in the secondary area but none of the effects that involved Time or Area was statistically significant (see Figure 6). Pain ratings of heat increased significantly in the primary area after HFS conditioning but remained stable in the secondary area (main effect for Time $F(1, 25) = 2.73$, $p = .111$; main effect for Area $F(1, 25) = 15.54$, $p = .001$; Time x Area interaction $F(1, 25) = 7.12$, $p = .013$) (see Figure 7). Investigation of the interaction indicated that, before conditioning, there was no significant difference between the pain ratings in the primary and secondary area ($t(25) = 0.83$, $p = .416$), but after conditioning, the pain rating in the primary area was higher than the rating in the secondary area (mean difference = 1.19, $SD = 1.74$, $t(25) = 3.49$. $p = .002$).

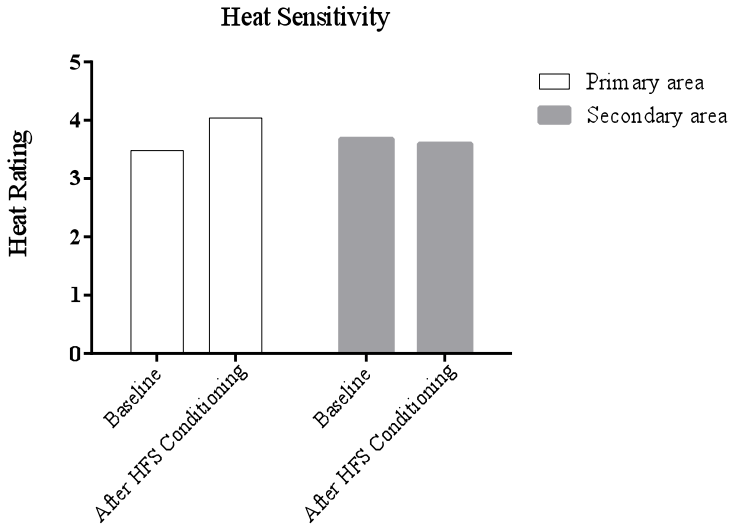


Figure 6. Heat rating in the forearm before and after HFS.

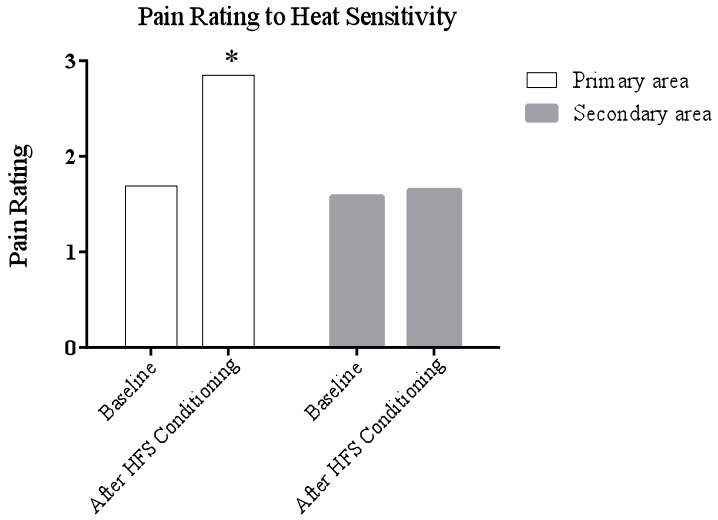


Figure 7. Pain rating to heat sensitivity in the forearm before and after HFS. *Pain rating to head sensitivity increased significantly after HFS only in the primary area.

Changes in Forehead Sensitivity after HFS Conditioning

Symmetry of forehead sensitivity before conditioning. No significant difference was observed in heat, sharpness, or pressure-pain sensitivity between the ipsilateral and contralateral (to the side of HFS conditioned forearm) sides of the forehead (see Appendix E).

Sharpness sensitivity to von Frey's monofilament. After conditioning, sharpness sensitivity to von Frey's monofilament decreased on both sides of the forehead but none of the effects that involved Time or Side was statistically significant (see Figure 8). Pain ratings to von Frey's monofilament also decreased on both side of the forehead but none of the effects that involved Time or Side was statistically significant (see Figure 9).

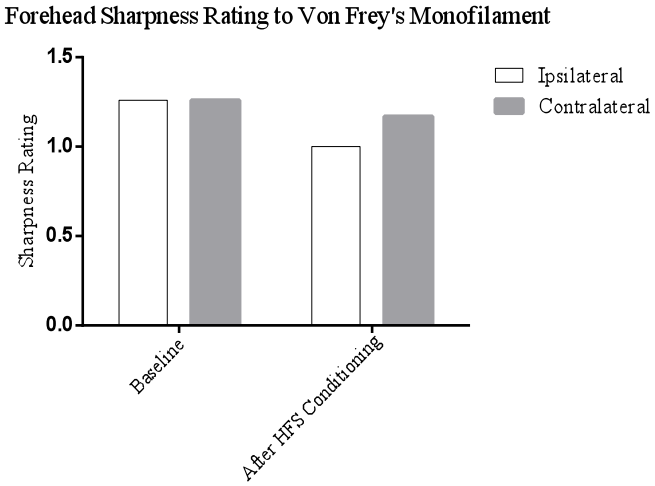


Figure 8. Sharpness rating to von Frey's monofilament on the forehead before and after HFS.

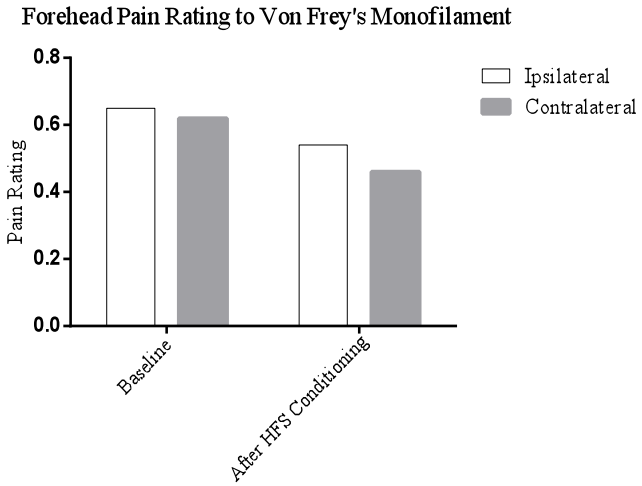


Figure 9. Pain rating to von Frey's monofilament on the forehead before and after HFS.

Sharpness sensitivity to pinprick. After conditioning, sharpness sensitivity to pinprick decreased on both sides of the forehead (main effect for Time $F(1,23) = 6.58$, $p = .017$; main effect for Side $F(1, 23) = 0.39$, $p = .54$; Time x Side interaction $F(1, 23) = 1.78$, $p = .195$) (see Figure 10). Pain ratings to pinprick also decreased after HFS conditioning on both sides of the forehead (main effect for Time $F(1, 23) = 4.90$, $p = .037$; main effect for Side $F(1, 23) = 1.00$, $p = .328$; Time x Side interaction $F(1, 23) = 0.033$, $p = .857$) (see Figure 11).

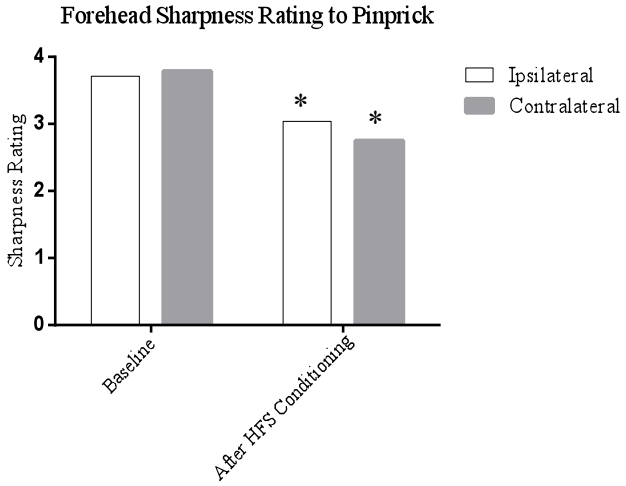


Figure 10. Sharpness rating to pinprick on the forehead before and after HFS. *Sharpness ratings to pinprick decreased significantly after HFS on both sides of forehead.

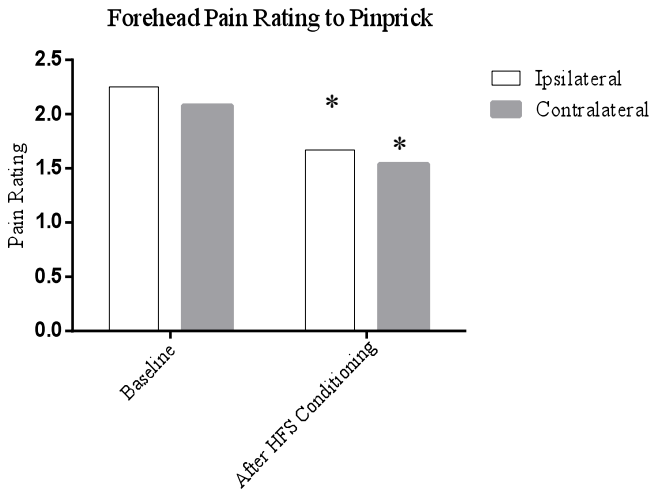


Figure 11. Pain rating to pinprick on the forehead before and after HFS. *Pain ratings to pinprick decreased significantly after HFS on both sides of forehead.

Pressure pain threshold (PPT). After conditioning, the PPT increased (i.e. the sensitivity to blunt pressure decreased) on both sides of the forehead but none of the effects that involved Time or Side was statistically significant (see Figure 12).

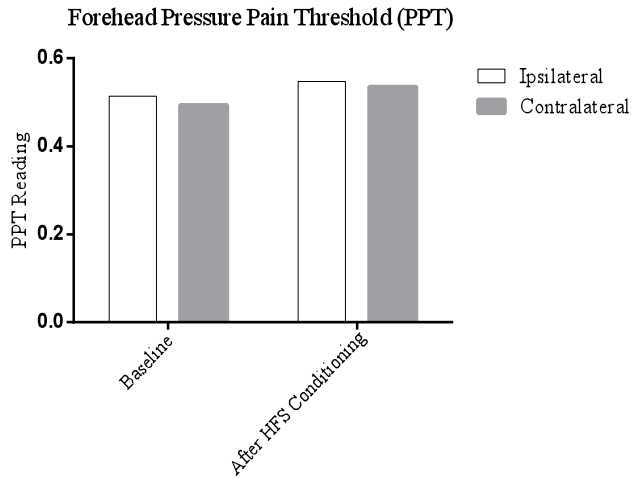


Figure 12. Pressure pain threshold changes on the forehead before and after HFS.

Heat sensitivity. After conditioning, heat sensitivity decreased on both sides of the forehead (main effect for Time $F(1, 25) = 10.91$, $p = .003$; main effect for Side $F(1, 25) = 0.17$, $p = .683$; Time x Side interaction $F(1, 25) = 3.03$, $p = .094$) (see Figure 13). Pain ratings to heat also decreased on both sides of the forehead (main effect for Time $F(1, 25) = 4.55$, $p = .043$; main effect for Side $F(1, 25) = 0.79$, $p = .381$; Time x Side interaction $F(1, 25) = 0.12$, $p = .731$) (see Figure 14).

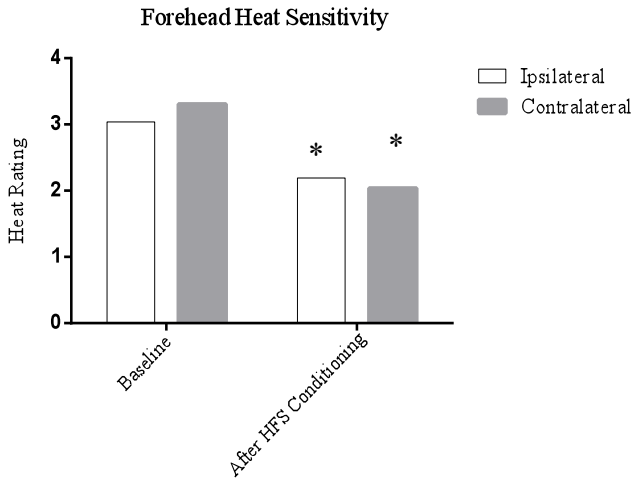


Figure 13. Heat rating on the forehead before and after HFS.
*Heat ratings decreased significantly after HFS on both side of forehead.

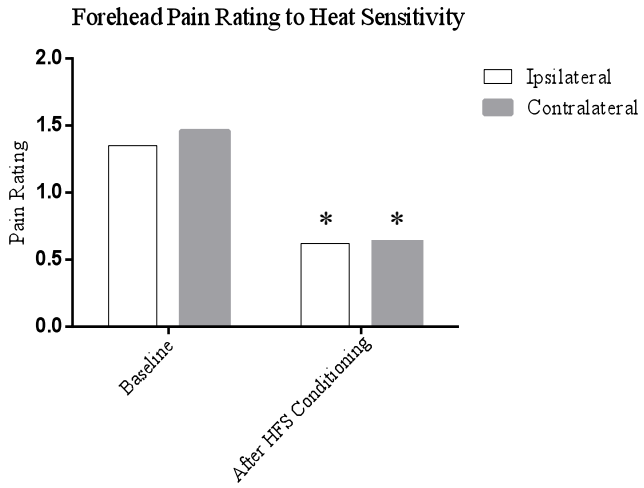


Figure 14. Pain rating to heat sensitivity on the forehead before and after HFS. *Pain ratings to heat sensitivity decreased significantly after HFS on both sides of forehead.

Changes in Foot Sensitivity after HFS conditioning

Sharpness sensitivity to von Frey's monofilament. After conditioning, sharpness sensitivity to von Frey's monofilament decreased on both feet (ipsilateral and contralateral to the HFS conditioned forearm) but none of the effects that involved Time or Side was statistically significant (see Figure 15). Pain ratings also decreased on both feet but none of the effects that involved Time or Side was statistically significant (see Figure 16).

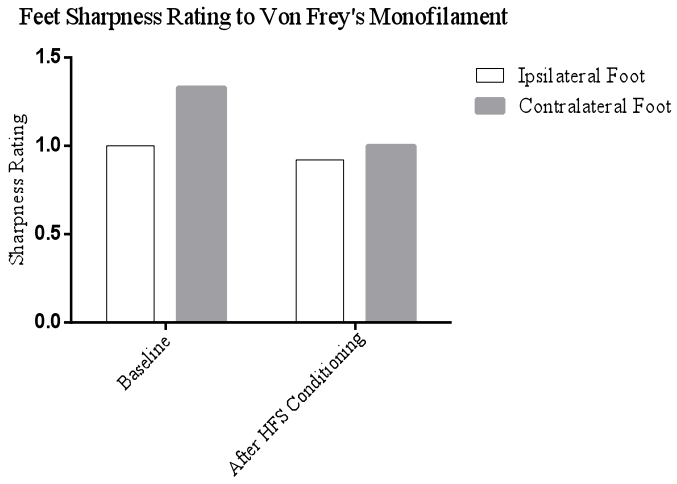


Figure 15. Sharpness rating to von Frey's monofilament on both feet before and after HFS.

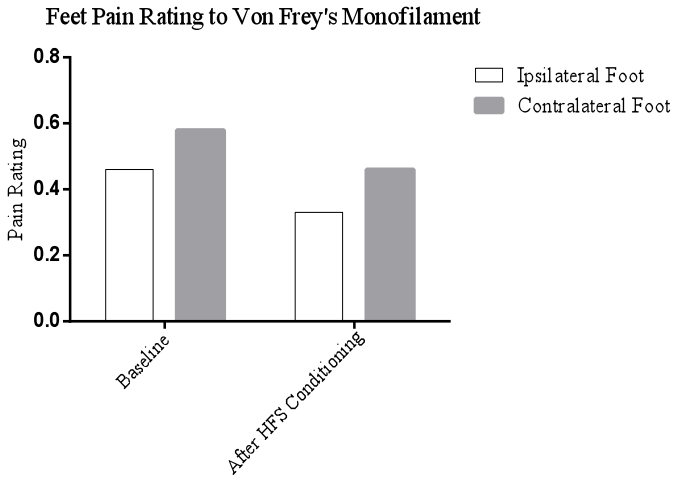


Figure 16. Pain rating to von Frey's monofilament on both feet before and after HFS.

Sharpness sensitivity to pinprick. After conditioning, sharpness sensitivity to pinprick decreased on both feet but none of the effects that involved Time or Side was statistically significant (see Figure 17). Pain ratings also decreased on both feet but none of the effects that involved Time or Side was statistically significant (see Figure 18).

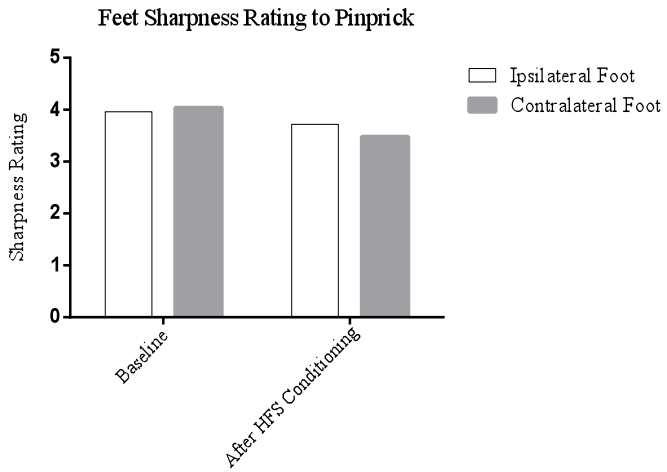


Figure 17. Sharpness rating to pinprick on both feet before and after HFS.

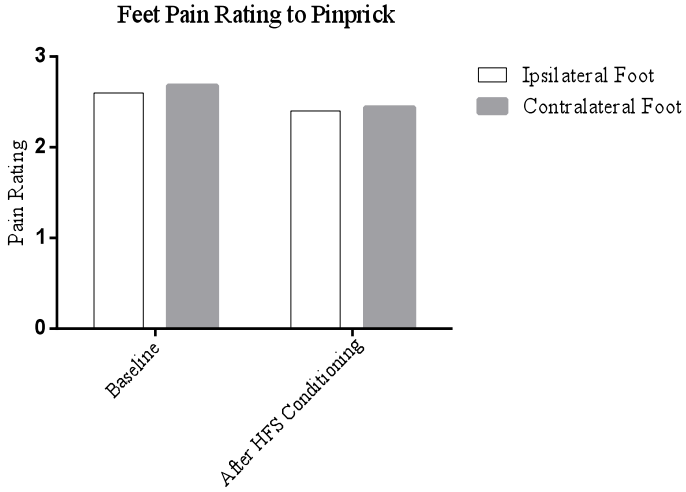


Figure 18. Pain rating to pinprick on both feet before and after HFS.

Pressure pain threshold (PPT). After conditioning, the PPT increased (i.e. the sensitivity to blunt pressure decreased) on both feet but none of the effects that involved Time or Side was statistically significant (see Figure 19).

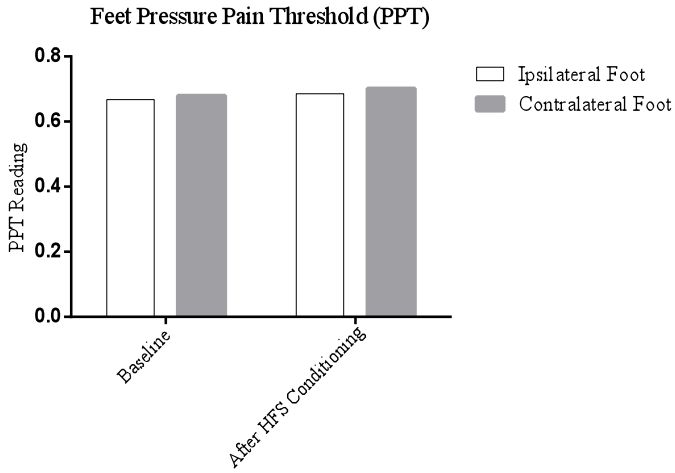


Figure 19. Pressure pain threshold changes on both feet before and after HFS.

Heat sensitivity. After conditioning, heat sensitivity decreased on both feet (main effect for Time $F(1, 25) = 7.41$, $p = .012$; main effect for Side $F(1, 25) = 0.09$, $p = .765$; Time x Side interaction $F(1, 25) = 0.57$, $p = .456$) (see Figure 20). Pain ratings also decreased on both feet (main effect for Time $F(1, 25) = 4.30$, $p = .049$; main effect

for Side $F(1, 25) = 0.30$, $p = .589$; Time x Side interaction $F(1, 25) = 0.00$, $p = 1.00$) (see Figure 21).

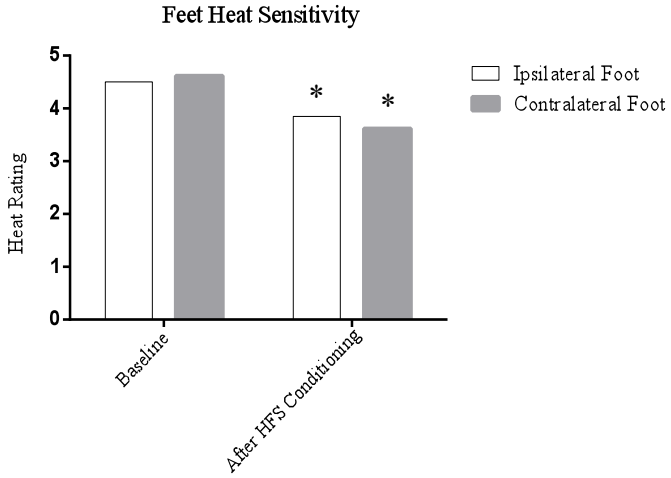


Figure 20. Heat rating on both feet before and after HFS.
*Heat ratings decreased significantly after HFS on both feet.

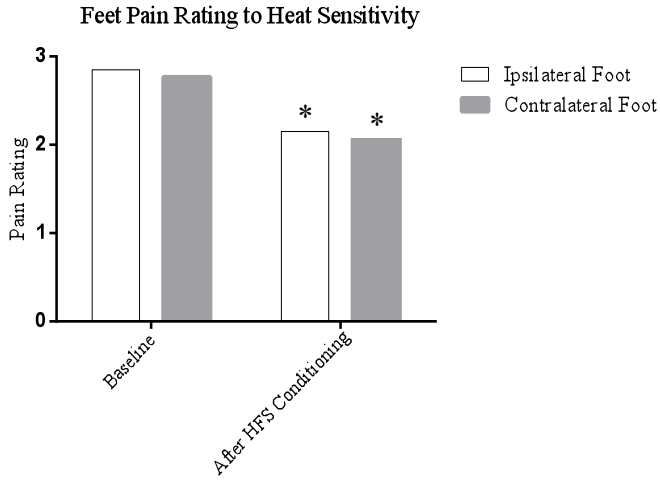


Figure 21. Pain rating to heat sensitivity on both feet before and after HFS.
*Pain ratings to heat sensitivity decreased significantly after HFS on both feet.

Pain Ratings to Supraorbital Stimuli

Pain ratings to supraorbital stimuli (blink reflexes elicited by large surface electrodes; ipsilateral or contralateral to the HFS conditioned forearm) decreased after HFS conditioning on both sides of the forehead but none of the effects that involved Time or Side was statically significant (see Figure 22).

Figure 22. Pain rating to supraorbital stimuli (blink reflexes elicited by large surface electrodes) before and after HFS.

R2 Latency

R2 latency differed significantly in relation to the laterality of supraorbital stimuli (ipsilateral or contralateral to supraorbital stimulation) but did not differ in relation to the laterality of HFS conditioning (ipsilateral or contralateral to HFS-conditioned side) before and after HFS conditioning (main effect for Side of supraorbital stimulation $F(1, 21) = 83.14$, $p < .001$; Side of supraorbital stimulation x Time interaction $F(1, 21) = 4.31$, $p = .05$; all other effects not significant) (see Figure 23). Investigation of this interaction indicated that, before HFS, latency_ii was shorter than latency_ic (mean difference = -3.01, $SD = 2.29$; $t(21) = -6.14$, $p < .001$), and also shorter than latency_cc (mean difference = -3.04, $SD = 1.52$; $t(21) = -9.39$, $p < .001$), but did not differ to latency_ci (mean difference = -0.02, $SD = 1.94$; $t(21) = -0.06$, $p = .957$). After HFS conditioning, latency_ii was also shorter than latency_ic (mean difference = -2.87, $SD = 2.03$; $t(21) = -6.64$, $p < .001$), and also shorter than latency_cc (mean difference = -2.78, $SD = 1.76$; $t(21) = -7.40$, $p < .001$), but did not differ to latency_ci (mean difference = -0.50, $SD = 2.09$; $t(21) = -1.11$, $p = .278$). *T*-test was also used to compare the latency changes between the ipsilateral side (to supraorbital

stimulation or HFS) before and after HFS, and between the contralateral side (to supraorbital stimulation or HFS) before and after HFS (mean difference of ii before and after HFS = 0.11, *SD* = 1.41, *t*(21) = 0.38, *p* = .71; mean difference of ic before and after HFS= 0.24, *SD* = 1.29; *t*(21) = 0.88, *p* = .39; mean difference of ci before and after HFS = -0.36, *SD* = 1.17, *t*(21) = -1.44 *p* = 0.17; mean difference of cc before and after HFS = 0.37, *SD* = 1.33, *t*(21) = 1.32, *p* = .20).

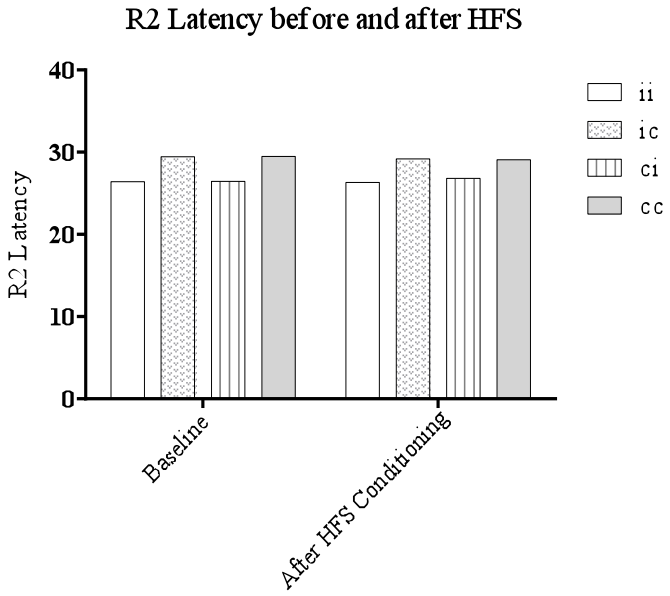


Figure 23. R2 latency of blink reflexes before and after HFS (ii = ipsilateral to both the HFS-treated site and supraorbital stimulus; ic = ipsilateral to HFS-treated site and contralateral to the supraorbital stimulus; ci = contralateral to the HFS-treated site and ipsilateral to the supraorbital stimulus; cc = contralateral to both the HFS-treated site and supraorbital stimulus).

R2 AUC

R2 AUC differed significantly in relation to the laterality of supraorbital stimuli but did not differ in relation to the laterality of HFS conditioning before and after HFS conditioning (main effect for Side of supraorbital stimulation $F(1, 23) = 103.31$, $p < .001$; all other effects were not significant) (see Figure 24). Investigation of this interaction indicated that AUC of the ipsilateral side (to supraorbital stimuli) was larger than the contralateral side (mean difference = 0.106, *SD* = 0.51; *t*(23) = 10.164, *p* < .001).

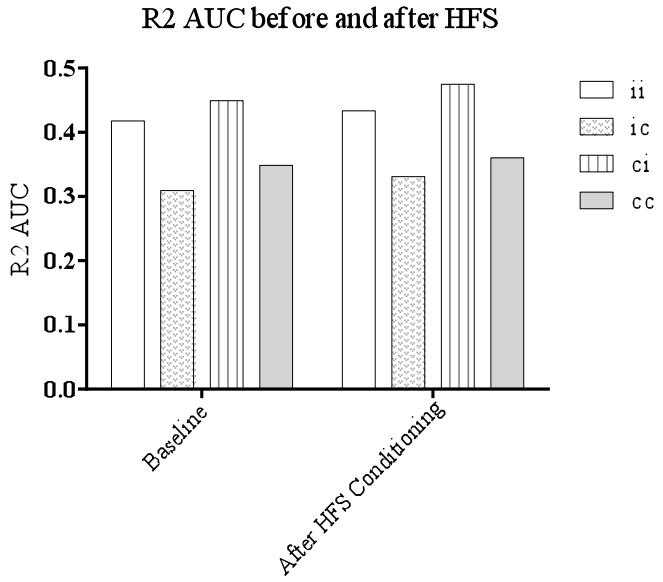


Figure 24. R2 rectified area under the curve (AUC) of blink reflexes before and after HFS.

Changes in R2 AUC after HFS conditioning. All R2 AUC were increased after HFS conditioning (mean increase_ii = 7.38%, *SD* = 0.21%; mean increase_ic = 9.17%, *SD* = 0.23%; mean increase_ci = 8.81%, *SD* = 0.26%; mean increase_cc = 9.08%, *SD* = 0.25%). However, after HFS conditioning, the changes in R2 AUC did not differ significantly either in relation to the laterality of supraorbital stimuli or to the laterality of HFS conditioning (see Figure 25).

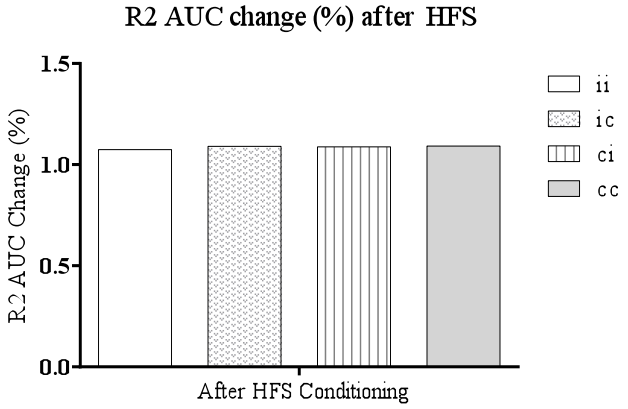


Figure 25. R2 AUC change after HFS conditioning expressed as the percentage of the R2 AUC at baseline (before HFS conditioning).

Discussion

Previous research found a dissociation between pressure-pain sensations and blink reflex excitability in the ipsilateral forehead after high frequency electrical stimulation (HFS) and the present study was an attempt to clarify these opposing findings by using a different form of supraorbital electrical stimulation. The first part of this study aimed to explore the effect of HFS on the changes in sensitivity to psychophysical stimulation in different areas of the body, and the second part aimed to explore the effect of HFS on the excitability of blink reflexes evoked by electrical stimulation at the supraorbital region. By comparing the outcomes from the first and the second part, we expected to obtain evidence to support our hypothesis that, by using large surface electrodes instead of concentric electrodes to elicit blink reflexes, there would be an agreement between pressure-pain sensations and blink reflex excitability in the ipsilateral forehead after HFS.

Changes in sensitivity to sharpness, heat, and pressure-pain before and after HFS on the forearm, forehead and both feet were compared, and the results partly supported the hypothesis that sensitivity would increase at the HFS conditioned test site but would decrease in an area remote from the conditioned site. On the other hand, when comparing the differences before and after HFS, neither the R2 onset latency nor the area under the curve (AUC) of blink reflexes had a significant change, which was contrary to our expectations. These findings suggest that the results failed to provide evidence to support our hypothesis that HFS would have an inhibitory effect on blink reflex activity. These results are now discussed.

Forearm Sensitivity Changes

Hypothesis 1: HFS would produce hyperalgesia (increase in sensitivity) to mechanical (sharp and pressure-pain) and heat stimuli in the primary area of the

conditioned forearm. The findings showed that both the sensation to von Frey's monofilament (mild sharpness) and its pain ratings increased significantly at the conditioned test site (primary area) after HFS. Similarly, heat and its pain ratings also increased at the conditioned test site after HFS, but only the pain ratings were significantly higher after HFS. However, although the sensation to pinprick (more intense sharpness) and its pain ratings also increased at the test site after HFS, these increases were not statistically significant. Similarly, when comparing pressure pain threshold (PPT) differences before and after HFS, there was a decrease (becoming more sensitive) at the test site after HFS but the change was not statistically significant.

In general, the results showed that the conditioned test site became more sensitive after HFS. Particularly, the increased sensitivity and pain ratings to von Frey's monofilament provide evidence consistent with the development of primary mechanical hyperalgesia. These results agree with previous research using this same conditioning technique (Lang, Klein, Magerl, & Treede, 2007; Vo & Drummond, 2012, 2013).

However, the present study did not demonstrate a clear development of primary hyperalgesia to heat stimulation. Some research (Ekblom & Hansson, 1987; Lang et al., 2007) using the same HFS conditioning technique also failed to observe heat hyperalgesia at the test site after HFS conditioning and they suggested that there could be a difference in impulse pattern in the fibres activated by the thermal stimulation as compared with the one activated by the pain stimulation (Ekblom & Hansson, 1987). In contrast, other studies have confirmed the development of primary hyperalgesia to heat sensation after HFS conditioning, just like the primary hyperalgesia to mechanical sensations (Culp et al., 1989; Hardy et al., 1950; Kilo et al., 1994; Raja et al., 1984; Vo & Drummond, 2012, 2013).

Why this effect varies across studies? One possible explanation could be due to the different approaches to elicit heat stimuli among different studies. For example, Lang et al. (2007) relied on the thermal threshold changes to determine the changes in heat sensitivity after HFS conditioning, such as, starting from 32 °C baseline temperature with 1 °C per second increase and they did not observe the development of primary hyperalgesia to heat stimuli after conditioning. On the other hand, some other studies (including the present study) keep the heat stimuli at a fixed but higher temperature, and rely on the feedback from the participants to determine whether there are changes in heat sensitivity after a certain conditioning. For example, Vo and Drummond (2013) used a 44 °C metal probe as the heat stimuli and they demonstrated primary heat hyperalgesia after HFS conditioning. Based on past research, gently warming the skin activates C-fibre thermoreceptors only, but painful heat stimuli with a higher temperature also activate both nociceptive A-delta and C-fibres (Hallin, Torebjork, & Wiesenfeld, 1982; Treede, Meyer, RAJA, & Campbell, 1995). Therefore, different ways of eliciting heat stimuli in fact activate different types of nerve fibres, thus generate different outcomes. As the present study also used the 44 °C metal probe to elicit heat stimuli, we expected to replicate findings of Vo and Drummond (2013) but we failed to do so (further details to explain the non-significant findings will be discussed in the methodological limitations section).

Interestingly, pain ratings to heat stimulation increased significantly after HFS conditioning. This might seem at odds with the non-significant primary heat hyperalgesia discussed above. However, there might be a possible reason to explain this. As described in the method section, a metal probe with 1.5 cm diameter was placed at the test site for seven seconds to access the heat sensation. When the metal probe was touching the skin, a mix of sensations was actually activated simultaneously: heat,

touch, and probably a blunt pressure as well (because a light force was needed to keep a good contact between the metal probe and the skin). Therefore, the pain ratings to heat might actually be the pain ratings to a mix of mechanical and thermal stimuli. Thus, the increased pain ratings might be providing extra evidence to support the development of the primary mechanical hyperalgesia. However, the present study only obtained a non-significant increase in the sensitivity to pinprick and pressure-pain at the test site after HFS, which is in contrast with previous research using the same conditioning technique (Lang et al., 2007; Vo & Drummond, 2012, 2013). To explain these non-significant findings, several possible reasons will be discussed later in the methodological limitations section.

Hypothesis 2: HFS would produce hyperalgesia to mechanical stimuli, but not to heat stimuli in the secondary area of the conditioned forearm. When investigating the sensation changes around the HFS conditioned test site (secondary area), the findings showed that the sensation to von Frey's monofilament and its pain ratings increased significantly after HFS. The sharpness to pinprick and its pain ratings also increased after HFS, but the increase was non-significant. Similarly, when comparing PPT differences before and after HFS, there was only a non-significant decrease after HFS. On the other hand, heat and its pain ratings remained stable after HFS. More important, when comparing the heat and its pain ratings between primary and secondary areas, both heat and the pain ratings in the primary area were significantly higher than those in the secondary area after HFS whereas the ratings were not significant different between these two areas before HFS.

These findings are important as they provide evidence of the development of secondary hyperalgesia (a sign of central sensitisation) to mechanical stimulation but not to heat stimulation, which is consistent with previous research using the same

conditioning technique (Klein et al., 2008; Lang et al., 2007; Raja et al., 1984; Vo & Drummond, 2012, 2013). Furthermore, it also provides evidence that the test site did become more sensitive to heat after HFS compared to the area adjacent to the conditioned test site, which actually differs from findings of Lang et al. (2007) that HFS fails to induce heat hyperalgesia at the conditioned site. However, similar to what was observed in primary area, the sensitivity to pinprick and pressure-pain did not change in the secondary area after HFS. In other words, the present study did not successfully induce secondary hyperalgesia to pinprick and pressure-pain, which is in contrast with previous research (Ali et al., 1996; Fuchs, Campbell, & Meyer, 2000; Vo & Drummond, 2012, 2013; Ziegler et al., 1999). More details to explain these non-significant findings will be discussed in the methodological limitations section.

Forehead Sensitivity Changes

Hypothesis 3: HFS conditioning would produce analgesia (decreased in sensitivity) to pressure-pain stimuli in the ipsilateral forehead. The findings showed that sensations to pinprick and heat as well as their pain ratings decreased significantly on both sides of the forehead after HFS. However, although sensation to von Frey's monofilament decreased and the PPT increased (becoming less sensitive) on both sides after HFS, the differences were not statistical significant.

The significant decrease in sensitivity to pinprick and heat stimulation might be due to the diffuse noxious inhibitory controls (DNIC) mechanism (Lautenbacher et al., 2002; Villanueva et al., 1986) or to stress-induced analgesia (Bandura, Cioffi, Taylor, & Brouillard, 1988; Gamaro et al., 1998; Kurrikoff, Inno, Matsui, & Vasar, 2008). The stress probably came from the painful electrical stimulation on the forearm (HFS conditioning) and also on the forehead (supraorbital stimulation) because the mean of the pain ratings were 5.21 (*SD* = 3.44) and 6.06 (*SD* = 2.04) respectively. The decreased

sensitivity is also in agreement with previous research that forehead analgesia has been observed after ice-induced, capsaicin-induced, or HFS-induced limb pain (Knudsen & Drummond, 2009, 2011; Vo & Drummond, 2012, 2013).

However, there is a contradiction compared to previous studies. The present study only observed a non-significant decrease in sensitivity to pressure-pain on both sides of the forehead after HFS and there was no significant difference of pressure-pain sensitivity between the ipsilateral and contralateral side of the forehead after HFS. In other words, the present study failed to observe analgesia to pressure-pain, especially on the ipsilateral side of the forehead after HFS, which has been clearly demonstrated in previous studies (Knudsen & Drummond, 2009, 2011; Vo & Drummond, 2012, 2013). For example, Knudsen and Drummond (2009) suggested that stress-induced analgesia might contribute to the bilateral forehead analgesia and in their study, as higher distress ratings were associated with greater forehead analgesia to pressure-pain. In another study, a bilateral decrease in sharpness ratings but an ipsilateral analgesia to pressure-pain was observed on the forehead after painful stimulation of the arm, possible due to dissociation between cutaneous (A-delta or C fibres; sharpness sensation) and deep (A-beta fibres; pressure-pain sensation) central pain pathways (Knudsen & Drummond, 2011). The present study failed to detect this dissociation as a bilateral decrease in sharpness was observed without ipsilateral analgesia to pressure-pain. One possible reason could be that, refer to appendix A (the timeline of test procedures), the first round blink reflex test was conducted before HFS conditioning, and the second-round psychophysical data was collected after HFS conditioning. Therefore, any changes (or the stability) of the psychophysical data after HFS might be partly due to the preceding supraorbital stimulation on the forehead (eliciting blink reflexes). In addition, the position of the electrodes for supraorbital stimulation on both sides of the forehead was

actually quite close to the area where the forehead psychophysical data was collected. In the present study, larger surface electrodes with a 7.5 mA current were used for the supraorbital stimulation in order to activate deeper nerve fibres. Therefore, it is possible that the supraorbital stimulation had successfully activated and facilitated deeper nerve fibres (A-beta), which in turn, neutralised the analgesic effect to pressure-pain caused by the HFS conditioning. In addition, compared with previous research (such as concentric electrodes with 2 mA used by Vo and Drummond (2012)), the supraorbital stimulation with the 7.5 mA current level was a stronger stimulation which was also painful for participants based on their feedback (more than half of them rated five or above in the 0-10 pain rating scale). Thus, after the painful supraorbital stimulation, primary and secondary hyperalgesia to mechanical stimulation may have developed at and surrounding the stimulated area. Therefore, it is possible that the hyperalgesia caused by supraorbital stimulation and the analgesia caused by DNIC from the HFS conditioned forearm happened together, which eventually cancelled each other. However, analgesia to pinprick on the forehead still developed after HFS, which might indicate that the supraorbital stimulation did not activate the cutaneous nerve fibres as much as the deeper nerve fibres. Because if it did, hyperalgesia to pinprick would be developed by the activated cutaneous nerve fibres and then we would not be able to observe the analgesic effect to pinprick on the forehead caused by HFS. Therefore, this might also provide further evidence that, as we expected, the supraorbital stimulation elicited by the large surface electrode with a higher current level did fulfil its purpose, which is to activate deeper nerve fibres instead of cutaneous nerve fibres.

Foot Sensitivity Changes

Hypothesis 4: HFS conditioning would produce analgesia to sharpness and pressure-pain stimuli on the ipsilateral foot. The findings showed that the sensation

to heat and its pain ratings decreased significantly on both feet but there was no significant difference between the ipsilateral and contralateral (to the conditioned forearm) foot. Although sensations to von Frey's monofilament and pinprick as well as their pain ratings all decreased after HFS, no statistical significant results were observed. PPT also increased in both feet after HFS, but the change was not statistical significant.

First of all, bilateral analgesia to heat sensitivity on the feet might be due to the DNIC mechanism (similar to the forehead analgesia to heat stimulation). Similar findings had been observed before. For example, Watanabe et al. (1996) found that, after healthy participants immersed their ipsilateral hand or foot in painful cold or painful hot water, there was a significant decrease in pain ratings to laser thermal stimulation applied to the leg indicating the development of analgesia to remote painful stimuli. However, contrary to previous findings, sensitivity to pinprick and pressure-pain did not change. Schliessbach et al. (2012) observed an ipsilateral analgesic effect to pressure-pain at the second toe after healthy participants immersed their hand in ice-saturated water. In another study, analgesia to capsaicin-induced and brush-evoked pain in the forearm was observed after healthy participants immersed the contralateral foot into painful cold water (Witting, Svensson, Arendt-Nielsen, & Jensen, 1998). Unfortunately, they did not investigate whether the analgesia effect was unilateral or bilateral. But Tuveson, Leffler, and Hansson (2006) identified a bilateral analgesia to pressure-pain on both thighs after tourniquet-induced ischemic pain in the left forearm, which can be viewed as the evidence to support that lower limb analgesia is a bilateral phenomenon. As discussed previously, any changes (or stability) of the second-round psychophysical data might be caused by both the supraorbital stimulation on the forehead and HFS conditioning on the forearm. Thus, one might expect that there

should be a stronger analgesic effect (no matter whether it is bilateral or ipsilateral effect) on the feet. It is not clear that why we still failed to observe a clear analgesic effect to pinprick and pressure-pain on both feet but more details will be discussed in the methodological limitations sections to explain these non-significant results.

Blink Reflex Changes

Pain rating changes before and after HFS. Pain ratings to supraorbital stimuli decreased after HFS on both sides of the forehead. This decrease is possibly also due to the diffuse noxious inhibitory controls or stress-induced analgesia discussed before and it corresponds with the analgesia to pinprick and heat sensation on both sides of the forehead. However, there was no significant difference of pain ratings in relation to the laterality of supraorbital stimuli or to the laterality of HFS conditioning.

R2 latency and AUC changes before and after HFS. The findings showed that R2 latency differed significantly only in relation to the laterality of blink reflexes but not to the laterality of HFS conditioned site. Before HFS and after HFS, latency of R2_ii (ipsilateral to both the HFS-treated site and supraorbital stimulus) was significant shorter than R2_ic (ipsilateral to the HFS-treated site and contralateral to the supraorbital stimulus), and R2_cc (contralateral to both HFS-treated site and supraorbital stimulus), but did not differ significantly from R2_ci (contralateral to the HFS-treated site and ipsilateral to the supraorbital stimulus). R2 latency (ii, ic, ci, and cc) did not change after HFS. Moreover, the findings also showed that, similar to R2 latency, R2 AUC differed significantly only in relation to the laterality of blink reflexes but not to the laterality of the HFS conditioned site.

These findings fail to provide any evidence to support our hypothesis that HFS would inhibit the R2_ii latency or R2_ii AUC. Together, the present study did not observe any effect of HFS conditioning on the blink reflex activity and it seems that the

activity of blink reflexes were predominately affected by the supraorbital stimuli only. However, in a similar study conducted by Vo and Drummond (2012), they found a dissociation between forehead ipsilateral analgesia to pressure-pain and ipsilateral facilitation of blink reflexes (elicited by concentric electrodes) after HFS conditioning. As discussed earlier, they attributed this dissociation to the distinct pain mechanisms between deep muscle pain evoked response (pressure-pain stimulation) and superficial pain evoked response (blink reflex stimulation). If the speculation of Vo and Drummond (2012) is true, HFS would have an inhibitory effect on blink reflexes (longer R2 latency or smaller R2 AUC) elicited by large surface electrodes as well as the forehead analgesia to pressure-pain because both of them are designed to test the activity of deep muscle nerve fibres. Unfortunately, neither a facilitatory nor inhibitory effect of HFS was observed in the present study.

Some factors might contribute to this non-significant finding. As discussed before, although the purpose of using large surface electrodes was to activate the deep A-beta nerve fibres, those electrodes also activated the superficial A-delta and C fibres simultaneously. Thus, the final outcome of blink reflex activity was actually a mixed result as both deeper muscle and superficial dermal nerve fibres had been activated at the same time. Therefore, it is not surprising to see that there is no obvious facilitatory or inhibitory effect of HFS conditioning on blink reflexes because it is highly possible that the facilitatory (to superficial fibres) and inhibitory (to deep muscle fibres) effects neutralised by each other. Another possible reason could be, as mentioned earlier, it seems that the blink reflexes were predominantly affected by the strong and painful supraorbital stimuli. Thus, it is possible that any effect of HFS conditioning might have been masked when both superficial and deeper nerve fibres had been lively activated by the supraorbital stimulation on the forehead.

Methodological Limitations

There are few major limitations in the present study. The first one is the reliance on self-report measures of sensation and pain ratings. In order to overcome this limitation, more objective measure of blink reflexes activity were employed.

Second, no matter which body area, the psychophysical tests failed to observe any significant hyperalgesia or analgesia to pressure-pain sensation. As discussed before, primary or secondary hyperalgesia to pressure-pain and forehead analgesia to pressure-pain have been clearly demonstrated after HFS conditioning in previous studies using similar testing equipment and settings. Thus, it was expected that we could replicate those findings. One possible reason that we failed to do so, might be due to the lack of familiarisation and practice before testing to allow more time for the experimenter to operate the algometer (to get the pressure pain threshold) more consistently, and also for the participants to learn how to accurately differentiate sensations from pressure to pressure-pain. Although a clear instruction was given to every participant prior to testing (such as "I will keep pushing this (the algometer) and please say 'stop' immediately when you start to feel the pain"), when certain force had been applied to skin, the feeling of pressure and the feeling of pain because of the pressure might be confusing for some of the participants if that was the first-time experience. They might have said stop too early when they were only feeling the pressure without pain, or they might have said stop too late when they took extra time to realise that they were actually feeling the pain. However, based on past research, pressure-pain sensation can be assessed reliably using algometers (Kinser, Sands, & Stone, 2009). Although in the present study, there were practices prior to the testing to get both experimenter and participants becoming familiar with all the test equipment, perhaps that was not enough. Therefore, in the future, it will be better if there is a

preliminary procedure (such as setting a certain period of time only for this purpose) for fresh experimenters and participants to have adequate familiarisation and practice prior the formal testing to ensure that more reliable testing data would be obtained. This limitation can also apply to other sensation tests, such as the von Frey's monofilament and pinprick tests as we also failed to replicate the hyperalgesia or analgesia effect on certain body areas. This might partly be due to the lack of adequate practice for the experimenter to operate the testing equipment in a reliable and consistent way. For example, if the experimenter couldn't apply the von Frey's monofilament to the participants' skin using a consistent force every time, the sharpness or pain ratings would be inconsistent regardless of the HFS conditioning effect. Therefore, the preliminary procedure would help the experimenter practice well and be able to operate the testing equipment in a reliable and consistent way.

Another major limitation comes from the testing sequence. As discussed above, the second-round psychophysical data was collected after the first-round blink reflexes test and HFS conditioning. Therefore, both HFS and supraorbital stimulation may have influenced the second-round psychophysical data. Thus, the final outcome was actually a mixed result. Also, according to the diffuse noxious inhibitory controls (DNIC) mechanism, there would be a decrease in sensitivity due to a remote painful stimulus. Thus, the painful supraorbital stimulation on the forehead might result a remote analgesic effect on the forearm and neutralise the hyperalgesia effect caused by HFS conditioning. This might be one of the reasons that why we could not observe the expected primary and secondary hyperalgesia to pinprick and pressure-pain sensations after HFS. Therefore, to avoid this confusion in the future, blink reflex tests should be separated from psychophysical tests. And, as suggested by past research (such as Vo and Drummond (2013)), the effect of HFS conditioning would persist for at least two

hours, thus, there should be at least several hours gap between psychophysical tests and blink reflex tests in order to ensure that no residual HFS effect from the first round test influences the second round outcome.

The last major limitation is coming from the imperfect way to use the large surface electrodes for the supraorbital stimulation. As discussed above, the large surface electrodes with a high current level would successfully activate deep muscle nerve fibres, but, unavoidably, the superficial nociceptive nerve fibres were also activated simultaneously. Therefore, it is still unknown whether HFS conditioning affects blink reflexes elicited by stimulation of deep muscle fibres. As suggested in previous study (Vo & Drummond, 2012), it would become a more objective and suitable way to correlate of the ipsilateral forehead analgesia to pressure-pain after HFS if we could prove that there is an inhibitory effect of HFS on the blink reflex activity stimulated only by deep muscle fibres. Based on past research (Kaube et al., 2000), one of the possible solutions to solve the problem, that large surface electrodes activate both superficial and deep muscle fibres at the same time, would be to apply the local anaesthetic cream on the supraorbital area to block the activity of superficial nerve fibres, such as the lidocaine/prilocaine cream 2.5% (EMLA cream) used in Kaube et al. (2000) study. As demonstrated in their study, the majority of A-delta and C-fibres were blocked by the superficial application of this EMLA cream. Therefore, in the future, this kind of cream could be used with large surface electrodes to elicit blink reflexes, so we will be able to observe the activity of deep muscle fibres without the inferences of the superficial nerve fibres.

Conclusions

On the one hand, the results of this study provide further evidence for understanding the underlying mechanisms (such as the diffuse noxious inhibitory

controls mechanism or the stress-induced analgesia) that influence healthy individuals' perception of pain. On the other hand, due to these major methodological limitations, the present study only partially supported the hypothesis that hyperalgesia would develop on the HFS conditioned forearm whereas analgesia would develop on the forehead and feet. No evidence was obtained to support the hypothesis that HFS conditioning would produce an inhibitory effect on the blink reflex activity. Thus, no corresponding result of HFS conditioning was observed between psychophysical response and blink reflex activity. However, with the suggestions discussed above, future studies may clarify issues raised in this study.

(Word count: 10,966)

References

Ali, Z., Meyer, R. A., & Campbell, J. N. (1996). Secondary hyperalgesia to mechanical but not heat stimuli following a capsaicin injection in hairy skin. *Pain, 68*, 401-411. doi:10.1016/S0304-3959(96)03199-5

Bandura, A., Cioffi, D., Taylor, C. B., & Brouillard, M. E. (1988). Perceived self-efficacy in coping with cognitive stressors and opioid activation. *Journal of Personality and Social Psychology, 55*, 479-488. doi:10.1037/0022-3514.55.3.479

Baumann, T. K., Simone, D. A., Shain, C. N., & LaMotte, R. H. (1991). Neurogenic hyperalgesia: The search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. *Journal of Neurophysiology, 66*(1), 212-227. Retrieved from http://0-jn.physiology.org.prospero.murdoch.edu.au/content/66/1/212.full.pdf+html

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & Van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology, 42*, 1-15. doi:10.1111/j.1469-8986.2005.00271.x

Blyth, F. M., March, L. M., Brnabic, A. J. M., Jorm, L. R., Williamson, M., & Cousins, M. J. (2001). Chronic pain in Australia: A prevalence study. *Pain, 89*, 127-134. doi:10.1016/S0304-3959(00)00355-9

Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B., & Touboul, C. (2008). Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain, 136*, 380-387. doi:10.1016/j.pain.2007.08.013

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain, 10*, 287-287. doi:10.1016/j.ejpain.2005.06.009

Costigan, M., & Woolf, C. J. (2000). Pain: Molecular mechanisms. *The Journal of Pain, 1*, 35-44. doi:10.1054/jpai.2000.9818

Culp, W. J., Ochoa, J., Cline, M., & Dotson, R. (1989). Heat and mechanical hyperalgesia induced by capsaicin. Cross modality threshold modulation in human C nociceptors. *Brain, 112*, 1317-1331. doi:10.1093/brain/112.5.1317

Ekblom, A., & Hansson, P. (1987). Thermal sensitivity is not changed by acute pain or afferent stimulation. *J Neurol Neurosurg Psychiatry, 50*(9), 1216-1220. Retrieved from http://0-jnnp.bmj.com.prospero.murdoch.edu.au/content/50/9/1216

Ellrich, J., Bromm, B., & Hopf, H. C. (1997). Pain-evoked blink reflex. *Muscle & Nerve, 20*, 265-270. doi:10.1002/(SICI)1097-4598(199703)20:3<265::AID-MUS1>3.0.CO;2-9

Ellrich, J., & Hopf, H. C. (1996). The R3 component of the blink reflex: Normative data and application in spinal lesions. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control, 101*, 349-354. doi:10.1016/0924-980X(96)95684-2

Ellrich, J., & Treede, R. D. (1998). Characterization of blink reflex interneurons by activation of diffuse noxious inhibitory controls in man. *Brain Research, 803*, 161-168. doi:10.1016/S0006-8993(98)00646-5

Fuchs, P. N., Campbell, J. N., & Meyer, R. A. (2000). Secondary hyperalgesia persists in capsaicin desensitized skin. *Pain, 84*(2–3), 141-149. doi:10.1016/S0304-3959(99)00194-3

Gamaro, G. D., Xavier, M. H., Denardin, J. D., Pilger, J. A., Ely, D. R., Ferreira, M. B. C., & Dalmaz, C. (1998). The effects of acute and repeated restraint stress on the nociceptive response in rats. *Physiology & Behavior, 63*, 693-697. doi:10.1016/S0031-9384(97)00520-9

Gatchel, R. J. (2004). Comorbidity of chronic pain and mental health disorders: The biopsychosocial perspective. *American Psychologist, 59*, 795-805. doi:10.1037/0003-066X.59.8.795

Giffin, N. J., Katsarava, Z., Pfundstein, A., Ellrich, J., & Kaube, H. (2004). The effect of multiple stimuli on the modulation of the 'nociceptive' blink reflex. *Pain, 108*, 124-128. doi:10.1016/j.pain.2003.12.014

Hallin, R. G., Torebjork, H. E., & Wiesenfeld, Z. (1982). Nociceptors and warm receptors innervated by C fibres in human skin. *J Neurol Neurosurgery Psychiatry, 45*, 313-319. doi:10.1136/jnnp.45.4.313

Hardy, J. D., Wolff, H. G., & Goodell, H. (1950). Experimental evidence on the nature of cutaneous hyperalgesia. *The Journal of Clinical Investigation, 29*, 115-140. doi:10.1172/JCI102227

Hitoto, T., Tsuruoka, M., Hiruma, Y., & Matsui, Y. (1998). A-delta afferent fiber stimulation activates descending noradrenergic system from the locus coeruleus. *Neurochemical Research, 23*, 1461-1465. doi:10.1007/BF03177488

Hopf, H. C. (1994). Topodiagnostic value of brain stem reflexes. *Muscle & Nerve, 17*, 475-484. doi:10.1002/mus.880170502

Human Anatomy. (2011). Types of nerve fibers. Retrieved from http://www.mananatomy.com/basic-anatomy/types-nerve-fibers

Julius, D., & Basbaum, A. I. (2001). Molecular mechanisms of nociception. *Nature, 413*, 203-210. doi:10.1038/35093019

Kakigi, R. (1994). Diffuse noxious inhibitory control. Reappraisal by pain-related somatosensory evoked potentials following CO2 laser stimulation. *Pathophysiology, 1*, 61. doi:10.1016/0928-4680(94)90147-3

Kaube, H., Katsarava, Z., Käufer, T., Diener, H. C., & Ellrich, J. (2000). A new method to increase nociception specificity of the human blink reflex. *Clinical Neurophysiology, 111*, 413-416. doi:10.1016/S1388-2457(99)00295-3

Kaube, H., Katsarava, Z., Przywara, S., Drepper, J., Ellrich, J., & Diener, H. C. (2002). Acute migraine headache: Possible sensitization of neurons in the spinal trigeminal nucleus? *Headache: The Journal of Head and Face Pain, 43*, 426-426. doi:10.1046/j.1526-4610.2003.03085_10.x

Kilo, S., Schmelz, M., Koltzenburg, M., & Handwerker, H. O. (1994). Different patterns of hyperalgesia induced by experimental inflammation in human skin. *Brain, 117*, 385-396. doi:10.1093/brain/117.2.385

Kimura, J., Daube, J., Burke, D., Hallett, M., Cruccu, G., Ongerboer de Visser, B. W., . . . Rothwell, J. (1994). Human reflexes and late responses. Report of an IFCN committee. *Electroencephalography and Clinical Neurophysiology, 90*, 393-403. doi:10.1016/0013-4694(94)90131-7

Kinser, A. M., Sands, W. A., & Stone, M. H. (2009). Reliability and validity of a pressure algometer. *Journal of Strength and Conditioning Research, 23*(1), 312-314. Retrieved from http://0-search.proquest.com.prospero.murdoch.edu.au/docview/213041284

Klein, T., Magerl, W., Hopf, H.-C., Sandkühler, J., & Treede, R. D. (2004). Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. *The Journal of Neuroscience, 24*, 964-971. doi:10.1523/jneurosci.1222-03.2004

Klein, T., Stahn, S., Magerl, W., & Treede, R. D. (2008). The role of heterosynaptic facilitation in long-term potentiation (LTP) of human pain sensation. *Pain, 139*, 507-519. doi:10.1016/j.pain.2008.06.001

Knudsen, L., & Drummond, P. D. (2009). Cold-induced limb pain decreases sensitivity to pressure-pain sensations in the ipsilateral forehead. *European journal of pain, 13*, 1023-1029. doi:10.1016/j.ejpain.2008.12.005

Knudsen, L., & Drummond, P. D. (2011). Cutaneous limb inflammation produces analgesia to pressure pain in the ipsilateral forehead of healthy volunteers. *The Journal of Pain, 12*, 451-459. doi:10.1016/j.jpain.2010.10.008

Kurrikoff, K., Inno, J., Matsui, T., & Vasar, E. (2008). Stress-induced analgesia in mice: Evidence for interaction between endocannabinoids and cholecystokinin. *European Journal of Neuroscience, 27*, 2147-2155. doi:10.1111/j.1460-9568.2008.06160.x

LaMotte, R. H., Shain, C. N., Simone, D. A., & Tsai, E. F. (1991). Neurogenic hyperalgesia: Psychophysical studies of underlying mechanisms. *Journal of Neurophysiology, 66*(1), 190-211. Retrieved from http://0-jn.physiology.org.prospero.murdoch.edu.au/content/66/1/190.full.pdf+html

Landon, D. N. (1976). *The peripheral nerve*. London: Chapman and Hall.

Lang, S., Klein, T., Magerl, W., & Treede, R. D. (2007). Modality-specific sensory changes in humans after the induction of long-term potentiation (LTP) in cutaneous nociceptive pathways. *Pain, 128*, 254-263. doi:10.1016/j.pain.2006.09.026

Lautenbacher, S., Roscher, S., & Strian, F. (2002). Inhibitory effects do not depend on the subjective experience of pain during heterotopic noxious conditioning

stimulation (HNCS): A contribution to the psychophysics of pain inhibition. *European Journal of Pain, 6*, 365-374. doi:10.1016/S1090-3801(02)00030-7

Magerl, W., Fuchs, P. N., Meyer, R. A., & Treede, R. D. (2001). Roles of capsaicin-insensitive nociceptors in cutaneous pain and secondary hyperalgesia. *Brain, 124*, 1754-1764. doi:10.1093/brain/124.9.1754

Melzack, R., Guite, S., & Gonshor, A. (1980). Relief of dental pain by ice massage of the hand. *Can Med Assoc J, 122*(2), 189-191. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1801755/pdf/canmedaj01126-0051.pdf

Melzack, R., & Wall, P. D. (1965). Pain mechanisms: A new theory: A gate control system modulates sensory input from the skin before it evokes pain perception and response. *Pain Forum, 5*, 971-979. doi:10.1016/S1082-3174(96)80062-6

Meyer, R. A., Ringkamp, M., Campbell, J. N., & Raja, S. N. (2005). Neural mechanisms of hyperalgesia after tissue injury. *Johns Hopkins Apl Technical Digest, 26* (1), 56-66. Retrieved from http://techdigest.jhuapl.edu/TD/td2601/Meyer.pdf

Rahman, W., D'Mello, R., & Dickenson, A. H. (2008). Peripheral nerve injury–induced changes in spinal α2-adrenoceptor–mediated modulation of mechanically evoked dorsal horn neuronal responses. *The Journal of Pain, 9*, 350-359. doi:10.1016/j.jpain.2007.11.010

Raja, S. N., Campbell, J. N., & Meyer, R. A. (1984). Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin. *Brain, 107*, 1179-1188. doi:10.1093/brain/107.4.1179

Rossi, B., Risaliti, R., & Rossi, A. (1989). The R3 component of the blink reflex in man: A reflex response induced by activation of high threshold cutaneous

afferents. *Electroencephalography and Clinical Neurophysiology, 73*, 334-340. doi:10.1016/0013-4694(89)90111-9

Saylor. (2011). Unit 4: The nervous system. Retrieved from http://www.saylor.org/site/wp-content/uploads/2011/01/TLBrink_PSYCH04.pdf

Schliessbach, J., van der Klift, E., Siegenthaler, A., Arendt-Nielsen, L., Curatolo, M., & Streitberger, K. (2012). Does acupuncture needling induce analgesic effects comparable to diffuse noxious inhibitory controls? *Evidence-Based Complementary and Alternative Medicine, 2012*, 1-5. doi:10.1155/2012/785613

Scholz, J., & Woolf, C. J. (2002). Can we conquer pain? *Nature Neuroscience, 5 Suppl 1*, 1062-1067. doi:10.1038/nn942

Schopflocher, D., Taenzer, P., & Jovey, R. (2011). The prevalence of chronic pain in Canada. *Pain Res Manag, 16*(6), 445-450. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3298051/pdf/prm16445.pdf

Sluka, K. A., Judge, M. A., McColley, M. M., Reveiz, P. M., & Taylor, B. M. (2000). Low frequency TENS is less effective than high frequency TENS at reducing inflammation-induced hyperalgesia in morphine-tolerant rats. *European Journal of Pain, 4*, 185-193. doi:10.1053/eujp.2000.0172

Sumikura, H., Andersen, O. K., Drewes, A. M., & Arendt-Nielsen, L. (2006). Secondary heat hyperalgesia induced by melittin in humans. *European Journal of Pain, 10*, 121-121. doi:10.1016/j.ejpain.2005.01.013

Tabachnick, B. G., & Fidell, L. S. (2007). *Experimental design using ANOVA*. Belmont, CA: Thomson, Brookes & Cole.

Téllez, M. J., Axelrod, F., & Kaufmann, H. (2009). The R3 component of the electrically elicited blink reflex is present in patients with congenital insensitivity to pain. *Pain, 141*, 178-180. doi:10.1016/j.pain.2008.10.030

Treede, R. D., Meyer, R. A., RAJA, S. N., & Campbell, J. N. (1995). Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. *J Physiol, 483*(Pt 3), 747-758. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1157815/pdf/jphysiol00327-0196.pdf

Tsuruoka, M., Hitoto, T., Hiruma, Y., & Matsui, Y. (1999). Neurochemical evidence for inflammation-induced activation of the coeruleospinal modulation system in the rat. *Brain Research, 821*, 236-240. doi:10.1016/S0006-8993(99)01133-6

Tsuruoka, M., Matsutani, K., & Inoue, T. (2003). Coeruleospinal inhibition of nociceptive processing in the dorsal horn during unilateral hindpaw inflammation in the rat. *Pain, 104*, 353-361. doi:10.1016/S0304-3959(03)00042-3

Tsuruoka, M., & Willis Jr, W. D. (1996). Bilateral lesions in the area of the nucleus locus coeruleus affect the development of hyperalgesia during carrageenan-induced inflammation. *Brain Research, 726*, 233-236. doi:10.1016/0006-8993(96)00410-6

Tsuruoka, M., & Willis, W. D. (1996). Descending modulation from the region of the locus coeruleus on nociceptive sensitivity in a rat model of inflammatory hyperalgesia. *Brain Research, 743*, 86-92. doi:10.1016/S0006-8993(96)01025-6

Tuveson, B., Leffler, A. S., & Hansson, P. (2006). Time dependant differences in pain sensitivity during unilateral ischemic pain provocation in healthy volunteers. *European Journal of Pain, 10*, 225-225. doi:10.1016/j.ejpain.2005.03.010

Verhaak, P. F. M., Kerssens, J. J., Dekker, J., Sorbi, M. J., & Bensing, J. M. (1998). Prevalence of chronic benign pain disorder among adults: A review of the literature. *Pain, 77*, 231-239. doi:10.1016/S0304-3959(98)00117-1

Villanueva, L., Peschanski, M., Calvino, B., & Le Bars, D. (1986). Ascending pathways in the spinal cord involved in triggering of diffuse noxious inhibitory controls in the rat. *Journal of Neurophysiology, 55*(1), 34-55. Retrieved from http://0-jn.physiology.org.prospero.murdoch.edu.au/content/55/1/34.full.pdf+html

Vo, L., & Drummond, P. D. (2012). *Coexistence of ipsilateral pain-inhibitory and facilitatory processes after high-frequency electrical stimulation*. Manuscript submitted for publication.

Vo, L., & Drummond, P. D. (2013). High frequency electrical stimulation concurrently induces central sensitization and ipsilateral inhibitory pain modulation. *European Journal of Pain, 17*, 357-368. doi:10.1002/j.1532-2149.2012.00208.x

Watanabe, S., Kakigi, R., Hoshiyama, M., Kitamura, Y., Koyama, S., & Shimojo, M. (1996). Effects of noxious cooling of the skin on pain perception in man. *Journal of the Neurological Sciences, 135*, 68-73. doi:10.1016/0022-510X(95)00253-X

Willer, J. C., Roby, A., & Bars, D. L. (1984). Psychophysical and electrophysiological approaches to the pain-relieving effects of heterotopic nociceptive stimuli. *Brain, 107*, 1095-1112. doi:10.1093/brain/107.4.1095

Willis, W. D. (2004). *Sensory mechanisms of the psinal cord* (3rd ed.). New York: Kluwer Academic/Plenum Publishers.

Witting, N., Svensson, P., Arendt-Nielsen, L., & Jensen, T. S. (1998). Differential effect of painful heterotopic stimulation on capsaicin-induced pain and allodynia. *Brain Research, 801*, 206-210. doi:10.1016/S0006-8993(98)00440-5

Woolf, C. J. (2004). Pain: Moving from symptom control toward mechanism-specific pharmacologic management. *Annals of Internal Medicine, 140*(6), 441-451. Retrieved from http://0-

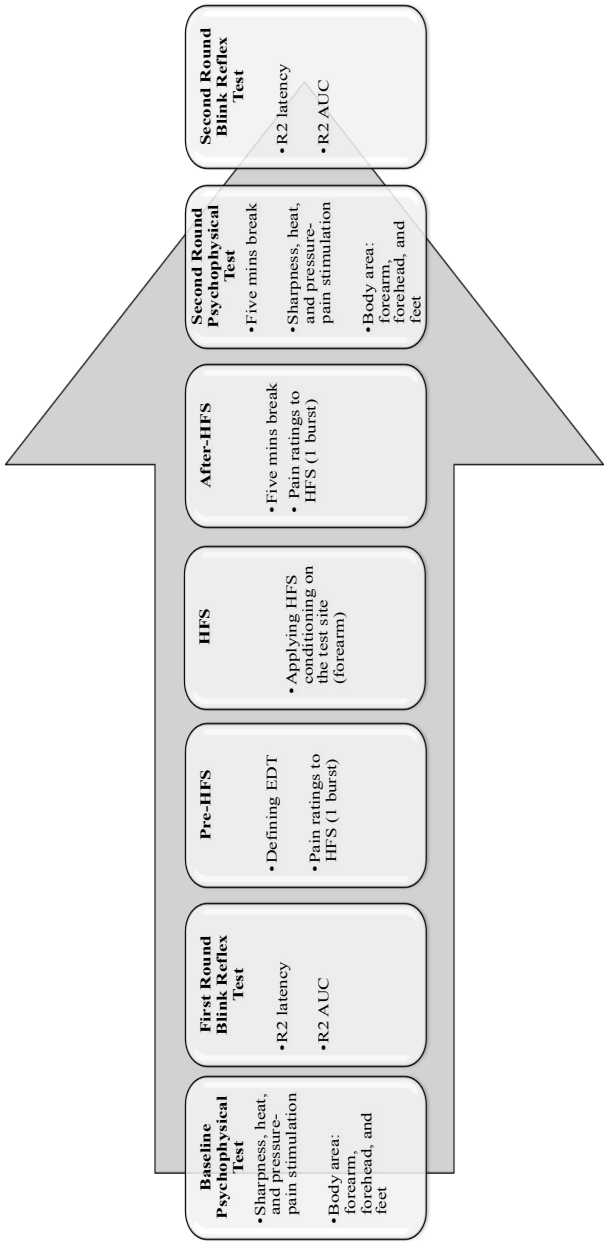
search.proquest.com.prospero.murdoch.edu.au/docview/222247811/fulltextPDF?accountid=12629

World Health Organization. (1946). Health. Retrieved from http://www.who.int/trade/glossary/story046/en/index.html

Ziegler, E. A., Magerl, W., Meyer, R. A., & Treede, R. D. (1999). Secondary hyperalgesia to punctate mechanical stimuli: Central sensitization to A-fibre nociceptor input. *Brain, 122*, 2245-2257. doi:10.1093/brain/122.12.2245

Appendix A

Timeline Depicting the Experimental Procedures



Appendix B

List of Removed Outliers Identified at the Preliminary Stage of SPSS Analysis

Variable	Total Number	Case Number
Forearm		
Mild Sharpness	2	8,11
Pain Ratings to Mild Sharpness	2	8,11
Pressure-pain Threshold	1	4
Forehead		
Mild Sharpness	3	9, 11, 23
Intense Sharpness	2	6, 11
Pain Ratings to Intense Sharpness	2	6, 11
Pressure-pain Threshold	2	4, 19
Foot		
Mild Sharpness	2	9, 11
Pain Ratings to Mild Sharpness	2	9, 11
Intense Sharpness	1	6
Pain Ratings to Intense Sharpness	1	6
Pressure-pain Threshold	1	4
Blink Reflex Test		
Latency	4	1, 4, 5, 19
AUC	2	17, 26
AUC Change (%)	1	26

Note. Mild Sharpness = Sharpness to Von Frey's Monofilament; Intense Sharpness = Sharpness to Pinprick.

Appendix C

F_{max} Test for Homogeneity of Variance

Variable	Largest Sample Variance	Smallest Sample Variance	F_{max}
Forearm			
Mild Sharpness	2.16	0.94	5.28
Pain Ratings of Mild Sharpness	1.02	0.66	2.39
More Intense Sharpness	2.23	1.99	1.26
Pain Ratings of More Intense Sharpness	2.07	1.25	2.74
Heat	1.96	1.63	1.45
Pain Ratings of Heat	2.47	1.8	1.88
Pressure-pain Threshold	0.32	0.26	1.51
Forehead			
Mild Sharpness	1.21	0.86	1.98
Pain Ratings of Mild Sharpness	0.94	0.71	1.75
More Intense Sharpness	2.35	1.92	1.50
Pain Ratings of More Intense Sharpness	1.75	1.31	1.78
Heat	1.13	1.96	0.33
Pain Ratings of Heat	2.23	1.29	2.99
Pressure-pain Threshold	2.53	1.82	1.93
Foot			
Mild Sharpness	1.31	0.78	2.82
Pain Ratings of Mild Sharpness	1.18	0.59	4.00
More Intense Sharpness	2.41	1.98	1.48
Pain Ratings of More Intense Sharpness	2.2	1.92	1.31
Heat	2.33	1.96	1.41
Pain Ratings of Heat	2.47	1.8	1.88
Pressure-pain Threshold	0.32	0.3	1.14
Blink Reflex			
Latency	2.3	1.66	1.92
AUC	0.17	0.12	2.01
Percentage Change of AUC after HFS	0.21	0.26	0.65

Note. Mild Sharpness = Sharpness to Von Frey's Monofilament; More Intense Sharpness = Sharpness to Pinprick; F_{max} = Largest Sample Variance^2/Smallest Sample Variance^2; According to Tabachnick and Fidell (2007), homogeneity of variance can be assumed when F_{max} is less than 10.

Appendix D

Summary of All the Non-Significant SPSS Results

	Main Effect for Time			Main Effect for Area			Time x Area		
	F	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Forearm									
Intense Sharpness	1.06	(1, 25)	.313	3.41	(1, 25)	.077	1.18	(1, 25)	.290
Pain Ratings to Intense Sharpness	1.03	(1, 25)	.320	1.26	(1, 25)	.273	1.32	(1, 25)	.262
Heat	0.74	(1, 25)	.397	1.21	(1, 25)	.282	3.26	(1, 25)	.083
Pressure_pain Threshold	2.23	(1, 24)	.148	3.65	(1, 24)	.068	0.00	(1, 24)	.993
	Main Effect for Time			Main Effect for Side			Time x Side		
	F	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Forehead									
Mild Sharpness	0.88	(1, 22)	.357	1.00	(1, 22)	.328	0.79	(1, 22)	.383
Pain Ratings to Mild Sharpness	0.80	(1, 25)	.381	0.68	(1, 25)	.416	0.074	(1, 25)	.788
Pressure_pain Threshold	1.70	(1, 23)	.205	0.85	(1, 23)	.366	0.033	(1, 23)	.858
Foot									
Mild Sharpness	1.72	(1, 23)	.203	2.19	(1, 23)	.153	1.13	(1, 23)	.299
Pain Ratings to MS	0.42	(1, 23)	.524	1.87	(1, 23)	.185	0.00	(1, 23)	1.00
Intense Sharpness	3.05	(1, 24)	.094	0.17	(1, 24)	.689	0.74	(1, 24)	.399
Pain Ratings to Intense Sharpness	0.69	(1, 24)	.414	0.15	(1, 24)	.700	0.01	(1, 24)	.923
Pressure_pain Threshold	0.26	(1, 24)	.618	0.17	(1, 24)	.688	0.004	(1, 24)	.950
Blink Reflex									
Pain Ratings to Supraorbital Stimuli	1.90	(1, 25)	.181	0.039	(1, 25)	.844	0.00	(1, 25)	1.00

	Main Effect for Side of Stimuli		
	F	*df*	*p*
Percentage Changes in AUC after HFS	0.21	(1, 24)	.653

Appendix E

Symmetry of Forehead Sensitivity before HFS Conditioning